ENVIRONMENTAL RISK ASSESSMENT OF EARLY LIFE STAGES OF WHITE STURGEON: METAL RELATED ISSUES

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ABSTRACT

Throughout North America populations of white sturgeon (*Acipenser transmontanus*) are threatened, in part due to poor annual recruitment. Definitive causes for this are not yet known, but the effects of contaminants are suspected to contribute. White sturgeon are exposed to a range of contaminants as they tend to inhabit industrialized river systems such as the Columbia and Fraser. White sturgeon are not commonly studied in ecotoxicology and their vulnerability as a species to contaminants of environmental concern is not well defined. To date, few exposure studies have been conducted with larvae, fry, and/or juveniles of this species; life stages often considered most susceptible to pollutants. Specifically, little work has been conducted to characterize effects of metals on white sturgeon.

In the Upper Columbia River (UCR) a population of white sturgeon has been experiencing poor annual recruitment for over thirty years, and the effects of metal pollution have been hypothesized as a potential contributing factor. In particular, Teck Metals Ltd. (Teck) operates a metallurgical facility in Trail, BC, Canada that currently discharges processed effluent into the river and historically released other metal containing tailings such as slag. There are concerns that concentrations of trace-elements, such as copper, lead, cadmium, and zinc, associated with the effluent and/or slag, might have detrimental impacts on the surrounding ecosystem, including the local white sturgeon population. In 2006, a remedial investigation and feasibility study (RI/FS) was initiated in the UCR, under the oversight of the US EPA, and this project is contributing to the portion dedicated to the risk assessment of the exposure of white sturgeon to metals.

The goals of this project were to develop information on toxicity of water, sediments and associated slag to help characterize sensitivity of white sturgeon to metals, and assess associated risks of metals on the population of white sturgeon in the UCR. Previous work conducted as part of a MSc degree, examined the effects of liquid effluent released by Teck on early life stages of white sturgeon. In addition, baseline information of toxicity due to sub-chronic exposure of early life stage sturgeon to copper, cadmium, and zinc, were developed. The thesis presented herein builds upon this previous work and has three major components to further characterize sensitivity of white sturgeon to metals and risk of exposure in the UCR. Specifically, a series of acute dose-response experiments were conducted with various early life stages of white sturgeon and resulting threshold values compared to water quality standards to assess protectiveness. Sensitivity of white sturgeon to metals was characterized by conducting parallel experiments with standard test species, such as rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*), as well as parallel field exposures in UCR water to develop water effect ratios (WERs) and assess relative bioavailability. A second set of experiments investigated whether exposure to water downstream of the metal smelter in Trail, BC affected survival or growth of white sturgeon. Mobile laboratories were situated riverside upstream and downstream of the smelter and the effects of potential contaminants within UCR water to early life stage white sturgeon were investigated under chronic exposure conditions. The third set of experiments involved characterizing UCR sediment toxicity and potential effects to sturgeon.

Results from this research indicate that early life stage white sturgeon are relatively sensitive to copper, cadmium, lead, and zinc in comparison to other fishes. Sturgeon were particularly sensitive to copper, especially during early life stage development when larvae are

transitioning to exogenous food. Thresholds for effects of copper on early life stage white sturgeon (LC_{50}'s ranged between 9 and 22 μg/L) bracket water quality criteria for the protection of aquatic life (7.9 μg/L ± 1.5). This result indicated that white sturgeon in the UCR might not be adequately protected. Environmentally relevant concentrations of metals, such as copper, found in water, sediment, or waters associated with sediment of the UCR, including pore water and overlying water, may approach or exceed water quality criteria and lethal concentration (LC) values for sturgeon. Results from the risk assessment portion of this project, however, indicated that contaminants in the water column downstream of the metal smelter at Trail did not likely affect survival of white sturgeon. Dilution of Teck effluent in the river is such that, at the major spawning site where early life stages of sturgeon are likely to be present and where the riverside experiments from the present project were conducted, there would be no toxicity expected. Contaminants associated with sediments in the UCR and their impact on survival of sturgeon is also of concern as early life stages inhabit benthic habitats, on surface sediments, or in interstitial space between stones. Analytical results from this project did indicate that UCR sediment downstream of the smelter facility were significantly greater ($p < 0.01$) in concentrations of trace-elements, such as copper, lead, cadmium, and zinc, relative to reference sites. However, survival of white sturgeon was not adversely affected following exposure to UCR sediments.

This project provided valuable information to help assess potential causes for poor recruitment of white sturgeon in the Columbia River. Advancements were made in characterizing the effects of metals to white sturgeon. In particular, life stage-specific sensitivities were identified that could have a significant impact on current risk assessment

approaches and the derivation of protective water quality standards. There are several hypotheses as to why the number of white sturgeon have been decreasing in the UCR over the last few decades, but as of yet, no definitive cause for poor recruitment has been identified. As more research is conducted, possible causes for recruitment failure can be eliminated. Based on results from this project, metals in the UCR do not appear to be contributing directly to decreased survival of early life stage sturgeon.

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
ATRF	Aquatic Toxicology Research Facility
AVS	Acid Volatile Sulfide
BBE	Birchbank Eddy
BC	British Columbia
BDL	Below Detection Limit
BERA	Baseline Ecological Risk Assessment
BLM	Biotic Ligand Model
CAS	Chemical Abstracts Service
CAS	Columbia Analytical Services
CB	China Bend
CI	Confidence Interval
CMC	Criteria Maximum Concentration
COI	Chemical Of Interest
COPC	Contaminants Of Potential Concern
CR	Columbia River
CTRL	Control
CWQG	Canadian Water Quality Guidelines
DDD	Dichlorodiphenyldichloroethane

DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DE	Deadman's Eddy
DME	Deadman's Eddy Sand Bar
DGT	Diffusive Gradients in Thin Film
DOC	Dissolved Organic Carbon
DFS	Downstream Field Site
DPF	Day Post Fertilization
DPH	Days Post Hatch
DQO	Data Quality Objective
DW	Dry Weight
EOT	End-Of-Test
EPA	United States Environmental Protection Agency
EqP	Equilibrium Partitioning
ESI	Environmental Standards Inc.
FAV	Final Acute Value
FM	Fathead Minnow
GE	Genelle
HDPE	High Density Polyethylene
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IUCN	International Union for Conservation of Nature
LA	Lethal Accumulation

LALL	Lower Arrow Lakes
LC	Lethal Concentration
LD	Little Dalles
LMF	Lower Marcus Flats
LOD	Limit of Detection
MDL	Method Detection Limit
MLE	Maximum Likelihood Estimate
mPECQ	Mean Probable Effect Concentration Quotient
MS-222	Tricaine Methanesulfonate
ND	Not Detected
NM	Not Measured
NP	Northport
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyls
PEC	Probable Effect Concentration
QA/QC	Quality Assurance/Quality Control
RI/FS	Remedial Investigation and Feasibility Study
RM	River Mile
ROW	Reverse Osmosis Water
RT	Rainbow Trout
SARA	Species At Risk Act
SD	Standard Deviation

SEM	Simultaneously Extracted Metals
SEMX	Excess Simultaneously Extracted Metals
SEMX, OC	Carbon-Normalized Excess Simultaneously Extracted Metals
SMAV	Species Mean Acute Value
SSD	Species Sensitivity Distribution
TAL	Target Analyte List
TEC	Threshold Effect Concentration
Teck	Teck Metals Ltd.
TOC	Total Organic Carbon
TU	Toxic Unit
UCR	Upper Columbia River
UCWSRI	Upper Columbia White Sturgeon Recovery Initiative
UFS	Upstream Field Site
UMF	Upper Marcus Flats
UofS	University of Saskatchewan
USEPA	US Environmental Protection Agency
WER	Water Effect Ratio
WQC	Water Quality Criteria
WQG	Water Quality Guidelines
WS	White Sturgeon

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Introduction

Populations of sturgeon (Acipenseridae) are threatened throughout the world, and over the past century populations of sturgeon have been diminishing in Northern Europe, Asia, and North America (Birstein 1993; Coutant 2004; Gisbert and Williot 2002). Within the last few decades there have been concerns about decreases in number of individuals in various populations and research and conservation efforts have increased. Factors such as age to reproductive maturity and an anadromous lifestyle make sturgeons particularly susceptible to changes in their environment. Over-harvesting and alteration of habitat, such as impoundment and pollution, are major factors that are hypothesized to have contributed to this decline (Birstein 1993; Gisbert and Williot 2002; Hu et al. 2009; Irvine et al. 2007; Luk'yanenko et al. 1999; Paragamian and Hansen 2008). Sturgeons are demersal fishes that spend much of their life in close association with the benthos, and this might result in exposure of all life stages to contaminants that are associated with sediments.

In North America, the white sturgeon (*Acipenser transmontanus*) is the largest fish to inhabit freshwater environments. Adults reach lengths of up to 6 metres and can weigh 800 kg. White sturgeon are extremely long lived and have been dated in excess of 80 years old. They are an archaic species with prehistoric ancestors dating back over a 100 million years (UCWSRI 2002). White sturgeon are anadromous fish and migrate from saltwater to freshwater environments during spawning events (Lebreton et al. 2004). In North American river systems, however, damming has altered the natural migration patterns of sturgeon, leading to

impoundment of certain populations. In north-western USA and British Columbia, Canada, populations of white sturgeon have been declining and this had been attributed primarily to poor annual recruitment (Coutant 2004; NRWSRI 2004; Scott and Crossman 1973, 1998; UCWSRI 2002). Without implementation of successful remedial efforts in the Columbia, Fraser, and Sacramento-San Joaquin rivers, the white sturgeon could become extinct in these waters within the next half century (Irvine et al. 2007; Paragamian et al. 2005; Paragamian and Hansen 2008; UCWSRI 2002).

A single causative factor for recruitment failure of white sturgeon has not been identified, and it is possible that a combination of stressors are contributing. Potential stressors include, among others: alteration of habitat, genetic bottlenecks or inbreeding depression, varying flow regimes, poor nutrition, predation by introduced species such as walleye (*Sander vitreus*), inter-specific competition, pathogens, decreased water quality, including temperature, turbidity, total dissolved gases, and pollution (Coutant 2004; Kruse and Scarnecchia 2002a; Kruse and Webb 2006; UCWSRI 2002). Little information currently exists in the literature on the effects of stressors to white sturgeon (Hildebrand et al. 2013), and further studies are needed to assess the relative sensitivity of this species to pollutants in comparison to other fishes (Foster et al. 2001a,b; Gundersen et al. 2008; Kruse and Scarnecchia 2002a,b; Kruse and Webb 2006; UCWSRI 2002; Webb et al. 2006). To date, most studies involving sturgeon have been conducted with yearlings or older, and few studies of effects of contaminants have been conducted with early life stages from embryo through juvenile (Vardy et al. 2011, 2013), life stages in fish that are often considered susceptible to toxicants (Hutchinson et al. 1998). There

are concerns about potential toxicity of contaminants such as metals to early life stages of white sturgeon (EPA 2006 a, b).

Fishes are particularly susceptible to metal toxicity in comparison to other animals. Metals are of particular concern in water bodies influenced by metallurgical activities, and are often found in contaminated systems at concentrations much greater than naturally occurring levels (Couillard et al. 1993; Kamunde and Wood 2003). The gill is typically the organ of first contact and the primary site of toxic action in most fishes exposed to aqueous metals (Playle et al. 1993a; Wood 2001), although recent findings have indicated effects on olfaction and mechanoreception can be sensitive endpoints (Grosell 2012). The dissolved forms of heavy metals are considered to be the most bioavailable and in turn most toxic to fishes (Wood 2001). The fish gill is a dynamic organ responsible for many critical functions, including gas exchange, ion exchange, acid-base regulation, and excretion of nitrogenous wastes (Wood 2001). Metals can disrupt these processes through interactions with proteins such as active transporters, ion channels, and specific enzymes. At elevated metal concentrations profound morphological changes in the gill can occur resulting in an inflammatory response that leads to suffocation (Wood 2012).

The blood of fishes is more or less concentrated in ions such as calcium (Ca), sodium (Na), potassium (K), and chlorine (Cl) compared to their environment depending on the osmolarity of the surrounding water. As a result, fish must actively regulate such ions across the gill to compensate for diffusion. Much research has focused around metal-gill interactions and it is widely accepted that certain metals such as copper (Cu), cadmium (Cd), lead (Pb), zinc (Zn),

and silver (Ag), to name a few, exert initial gill toxicity by disrupting ionic regulation and homeostasis (Playle et al. 1993a; Wood 2001, 2012). Metals ions compete with nutritive ions for uptake in pavement cells, and once inside, inhibit ATP-dependent enzymes (ATPase's) on the basolateral surface of the cell (Wood 2012). This prevents the active uptake of the nutritive ions into the blood, which is critical to offset the passive losses of ions such as Na^+, Cl^-, and Ca^+ in freshwater fishes. This eventually results in a collapse of the circulatory system due to extensive perturbations of the osmotic gradient throughout the fish (Wood 2012).

Copper, for example, competes with Na^+ at the epithelial Na channel (ENaC) located on the apical membrane and impairs uptake. Evidence that Cu enters via the Na channel in competition for Na is provided by Handy et al. (2002) who explains that these ions have similar mobilities (λ) in solution (λ, Na^+, 50.1; Cu^{2+}, 53.6 cm^2 Int. $ohms^{-1}$ $equiv^{-1}$) and that Cu^+ (after hypothesized reduction of Cu^{2+} to Cu^+ at the cellular surface) may compete equally with Na^+ for diffusion to membrane surfaces. Once inside the cell, Cu inhibits Na^+/K^+ -ATPase at the basolateral membrane and prevents Na^+ from entering the blood (Pelgrom et al. 1995; Li et al. 1996; Wood 2001; Grosell and Wood 2002; Kamunde and Wood 2003). Na^+/K^+ -ATPase is rich in sulfhydryl groups (SH-) and it is thought that Cu, with its high affinity for thiols, binds to these catalytic groups and inhibits pump function (Pelgrom et al. 1995; Li et al. 1996; Wood 2001).

White sturgeon might be at increased risk of exposure to metals as they tend to inhabit both the water column and the benthos. Previous work indicated that early life stages of white sturgeon are relativity sensitive to certain metals, such as Cu, during chronic aqueous exposures

(Vardy et al. 2011). However, little toxicity data currently exists in regards to sturgeon sensitivity to metals, especially through exposure routes such as sediment. In particular, the larval and early juvenile stage where fry are in proximity to sediments, and to contaminants such as metals that are associated with sediments, is of interest. Early life stages of sturgeon inhabit benthic habitats, on surface sediments, or in interstitial space between stones, and yolksac stage white sturgeon tend to hide/burrow in refugia (Brannon et al. 1983, 1985; Gessner et al. 2009; McAdam 2011; Richmond and Kynard 1995; personal observation in the laboratory). Consequently, more toxicity studies with white sturgeon and metal exposures are needed.

1.2 Contaminants and the Columbia River

The Columbia River is an ideal system to examine white sturgeon biology and the potential stressors that may be contributing to their decline. The Columbia River borders both Canada and the United States and is the largest river in western North America (Figure. 1.1). With fourteen major dams and several hundred smaller dams, the Columbia River produces vast amounts of hydroelectric power and is the most hydroelectrically developed river system in the world (CCRH 2008). Damming, however, has complicated migration for several species of fish, including salmon (Salmonidae) and white sturgeon, that utilize the river and its tributaries as spawning grounds. The incorporation of fish locks have helped certain fishes bypass the dams but often prove ineffective for larger species such as sturgeon. As a result, the river is home to several impounded populations of white sturgeon that are experiencing varying degrees of recruitment failure (Coutant 2004). Anthropogenic impacts can alter riverine ecosystems and there are concerns over the impacts on sturgeon in the Columbia River (Hildebrand et al. 2013).

As of 2006, the federal government of Canada listed white sturgeon as endangered under the Species At Risk Act (SARA).

Figure 1.1. Columbia River basin.
Image from K. Musser; http://en.wikipedia.org/wiki/File: Columbiarivermap.png.

A population of white sturgeon in the Upper Columbia River (UCR) that resides between Hugh L. Keenleyside Dam in southern British Columbia, Canada (Figure 1.2) and Grand Coulee Dam in Washington State USA (Figure 1.3) is of particular interest and has been experiencing poor annual recruitment for over forty years (Hildebrand et al. 1999). Recent investigations suggest that this population is comprised primarily of ageing adults with potentially less than

2000 individuals (Hildebrand et al. 2013). Studies have found evidence that females are spawning in areas of the river considered to be suitable habitats, such as Waneta Eddy located near the Pend'Oreille River-Columbia River confluence (Figure 1.2), and that fertilization of viable eggs is occurring in certain locations (Golder Associates Ltd., 2007; Howell and McLellan, 2006). However, very few young of the year have been subsequently found in the river. As part of recovery efforts, the Upper Columbia River White Sturgeon Recovery Initiative (UCWSRI) was formed into 2000 and has been releasing hatchery raised yearlings into the river. After release these hatchery fish display good survival, growth, and body condition (Hildebrand et al. 2013). Thus, for unknown reasons, this population of white sturgeon in the UCR is experiencing a life stage-specific bottleneck.

The potential for exposure to metals and other contaminants in the Columbia River is of concern due to past and present activities of mines, metallurgical facilities, pulp and paper mills, sewage treatment plants, as well as other industrial and municipal sources (UCR WPRIFS 2008). Of particular concern are the effects of a large metal smelter operated by Teck Metals Ltd. (Teck), located in Trail, BC, Canada (Figure 1.2). As a result of their operations, Teck discharges processed effluent into the Columbia River. The effluent contains various contaminants, including trace amounts of zinc, mercury, copper, ammonia, arsenic, cadmium, chlorine, and lead (Teck American Incorporated 2008). In addition, Teck historically released slag into the river. It is estimated that approximately 12 million metric tons of slag were released between 1930 and 1995 (Teck American Incorporated 2008; Fairchild et al. 2012). Slag is composed of ferrous granules that are relatively dense and tend to accumulate on the river bottom. There is concern over the toxicological properties, such as leaching of metals, from slag and the potential

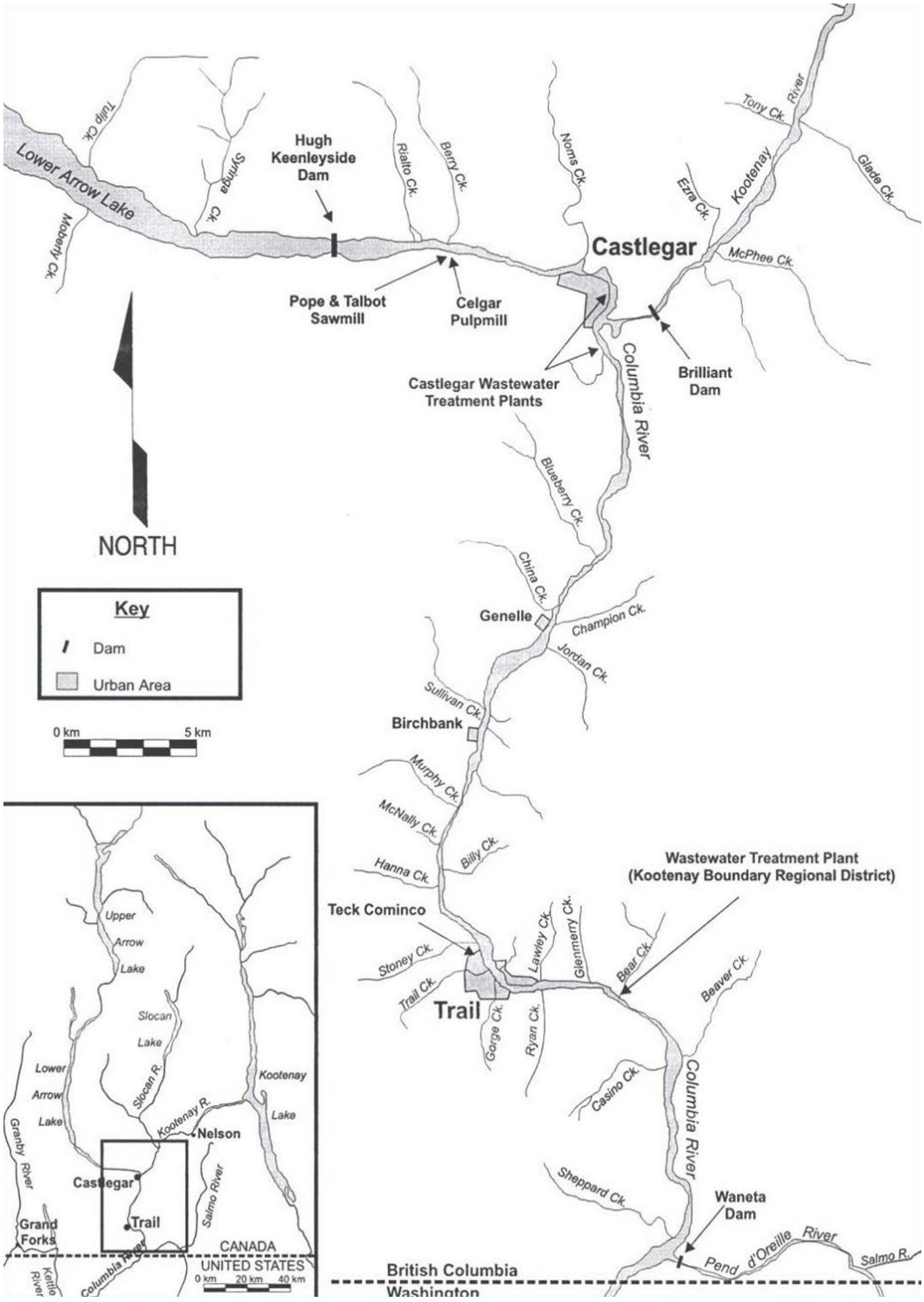

Figure 1.2. Canadian portion of the distribution range of a population of white sturgeon that resides between Hugh L. Keenleyside Dam, BC, CA and the Grand Coulee Dam, WA, USA.

Image from Golder Associates, Castlegar, BC.

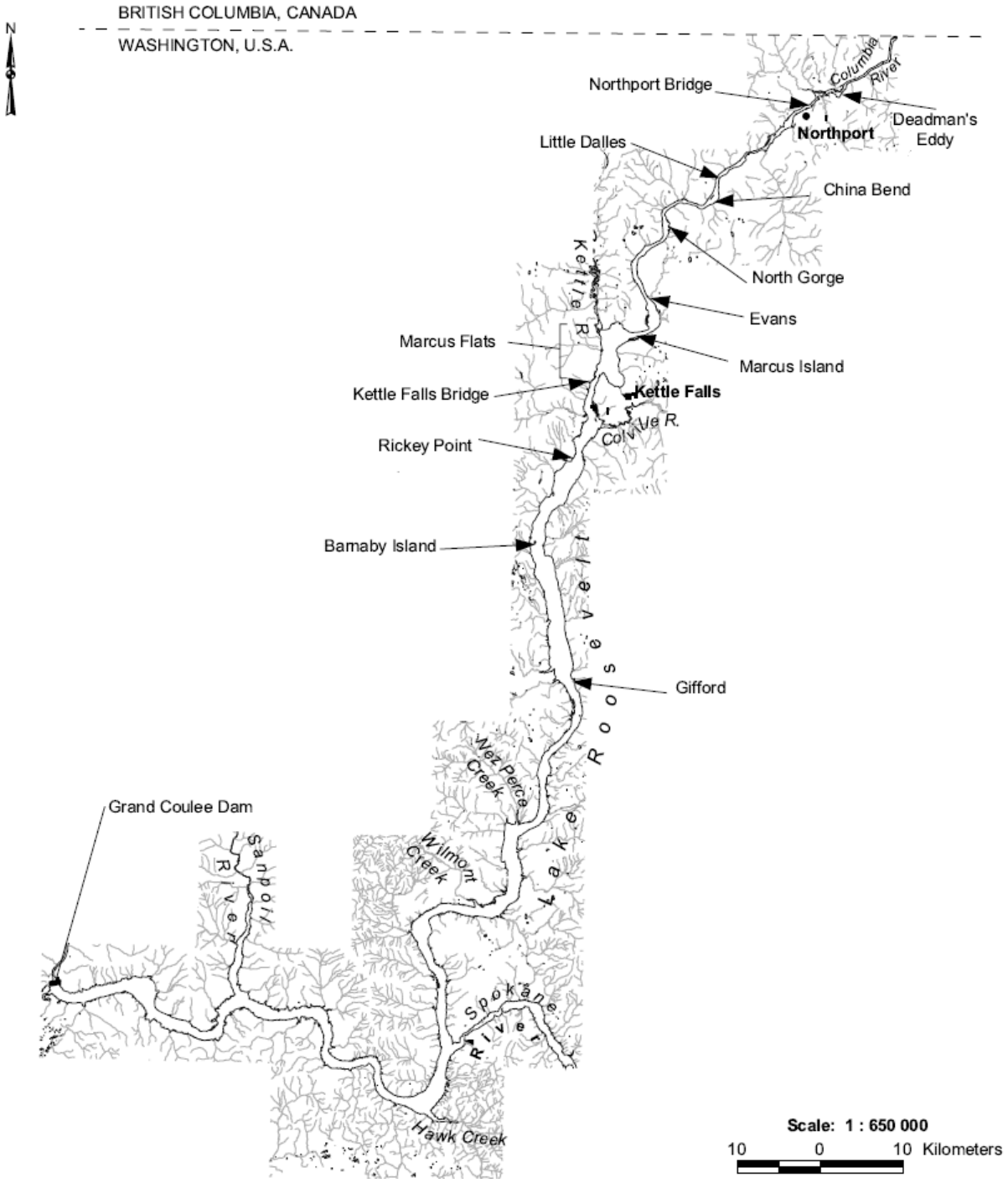

Figure 1.3. Washington portion of the distribution range of a population of white sturgeon that resides between Hugh L. Keenleyside Dam, BC, CA and the Grand Coulee Dam, WA, USA.

Image from Golder Associates, Castlegar, BC.

impact on white sturgeon (EPA 2006 a, b). Elevated concentrations of metals have been documented in sediments of the Columbia River in comparison to background levels and might be of ecological significance (Bortleson et al. 2001; EPA 2006 a,b; Johnson 1990, 1991). It has been suggested that under certain hydrological conditions metals might leach from sediments of the Columbia River into porewater and overlying water (Paulson and Cox 2007), and that benthic invertebrates might be adversely affected (Besser et al 2008.). In 2006, a remedial investigation and feasibility study (RIFS) was initiated in the UCR to investigate the potential impact of contaminated water and sediment in the environment and the associated risks (EPA 2006 a,b).

Currently, a definitive method for development of sediment quality guidelines (SQGs) does not exist and there is no clear technique that stands alone; all have their strengths and weaknesses. There are numerous approaches that vary in the procedures, the amount and type of data required, the statistical analyses employed, and the variables considered, but in general they can be grouped into three main categories: empirical, mechanistic, and consensus based approaches (Wenning et al. 2005). Empirical approaches compare chemical concentrations and biological effects in field and laboratory data utilizing large databases and statistical analyses (Burnett-Seidel 2011), whereas mechanistic approaches are theoretically based and designed to predict sediment toxicity based on an understanding of the chemical and variables that influence toxicity (Wenning et al. 2005). Mechanistic approaches account for differences in bioavailability of contaminants through equilibrium partitioning (EqP) in the interstitial water (Burton 2002). Consensus based approaches combine previously established guidelines that used different methods of derivations but produced similar results and generate new SQGs from their central

tendencies (Wenning 2005). Some of the more common methods and tools used in deriving SQGs include: the sediment background approach, the spiked-sediment toxicity test, utilizing water quality guidelines, interstitial water toxicity and the equilibrium partitioning approach, examining tissue residues, benthic community structure assessment and the screening level concentration approach, the sediment quality triad, the apparent effect threshold approach, the effects range approach, the threshold and probable effect levels approach, and more recently, the consensus approach, the field-based species sensitivity distribution approach, and regression-type approaches.

In Canada, the Canadian Council of Ministers of the Environment (CCME) has implemented protocols for the development of national SQGs that are based on a modification of the National Status and Trends Program (modified NSTP) approach and the spiked-sediment toxicity test (SSTT) approach (CCME 2001). The NSTP approach uses co-occurrence data (measured concentrations of chemicals in sediment and associated biological effects) to develop a threshold effect level (TEL) and a probable effect level (PEL). The TEL is a concentration below which adverse effects are not expected to occur and is consistent with the Canadian SQG (CCME 2001). The SSTT approach involves controlled laboratory spiked-sediment toxicity tests with benthic organisms and attempts to provide cause-effect relationships and information on environmental parameters that may influence bioavailability and toxicity. Generally, the lower value that is derived from the two approaches would be used in the SQG, but to date little SSTT data exist that meet the minimum requirements that have been set by the CCME as standard methodologies are still being developed. As a result, interim sediment quality guidelines (ISQGs) are implemented if only one approach is used until further data is gathered. In Canada, it is the

intention that the SQGs be used in conjunction with other supporting information, such as background levels, concentrations of other naturally occurring substances, biological assessments, environmental quality guidelines of other media, and other sediment quality assessment values of other chemicals (CCME 2001). Other approaches that are used in Canada, such as Ontario, include the EqP approach that establishes no-effect levels (NELs), and the screening-level concentration (SLC) approach that establishes low-effect levels (LELs) and sever-effect levels (SELs; Burnett-Seidel 2011; Persaud et al. 1993). Approaches used in the United States include the EqP and SLC method, as well as a variety of co-occurrence data methods (Burnett-Seidel 2011). In the present project a variety of these approaches and tools are utilized to help characterize risk to white sturgeon in the Columbia River.

The purpose of this project was to build upon previous work that was conducted in fulfillment of a Masters thesis (Vardy D. 2011. MSc Thesis) and further address the potential impact of exposure to metals on early life stages of white sturgeon. More investigation into contaminants and their effects on sturgeon in the natural environment is needed. Difficulties arise when interpreting and translating results of laboratory studies to field conditions. This is particularly true for sturgeon that have lifecycles that span several decades in differing habitats, making environmentally relevant studies difficult to conduct. Field experiments are valuable as they have the advantage of incorporating multiple variables within an environment that may influence an organism's sensitivity to a particular stressor. For this project, a metal related risk assessment of sturgeon focusing on the effects of metals in the UCR was conducted. Acute exposures were performed in both the laboratory and in the field to characterize sturgeon sensitivity to metals at various life stages and under UCR specific water quality conditions.

Furthermore, chronic riverside field studies were conducted to investigate the potential toxicity of UCR whole water to sturgeon. In addition, chronic laboratory studies were conducted to investigate the potential effects of contaminated sediments from the UCR to early life stage white sturgeon. These chronic studies allowed for a more accurate assessment of the potential interaction and effect of multiple contaminants that might influence toxicity to sturgeon in the UCR. In the environment, contaminants such as metals typically occur as mixtures (Fairbrother et al. 2007) and exposure to field samples help to characterize site specific toxicity.

Metal bioavailability dictates toxicity and factors such as organic matter, suspended particles, and redox status within a particular matrix can vary greatly between environments and needs to be evaluated when assessing risk. When developing site specific water quality standards for metals, threshold values are typically adjusted for hardness (CCME 2003; Stephan et al. 1985). In certain cases, toxicity tests may be conducted in site specific water in order to develop water effect ratios (WERs) for greater specificity in developing standards. Logistically, however, this is not always feasible and can be costly. The bioavailability of a metal is largely determined through its speciation, with the ionic species of metals typically considered most toxic (Wood 2012). Factors such as water hardness, pH, alkalinity, and organic complexes play a crucial role in metal bioavailability (Playle et al. 1993a; Wood 2001). The biotic ligand model (BLM) is a well-documented metal speciation and predictive toxicity model used extensively in assessing metal toxicity to fishes (Niyogi and Wood 2004; Paquin et al. 2002). The basic concepts of metal-gill interactions, as previously described for a few heavy metals, forms the basis of the BLM. The BLM utilizes the physiochemical properties of the gill and the associated interactions with metals and ambient water quality parameters to predict metal toxicity to fish in the

environment (Paquin et al. 2002). Certain cations compete with heavy metals for receptors sites on an organism's "biotic ligand", the biologically sensitive receptor such as the gills, which is considered to be the site of toxic action when considering the BLM framework (BLM 2007). In addition, organic and inorganic ligands can complex with metals while varying water quality parameters can change metal speciation, both affecting bioavailability. The BLM accounts for these properties and predicts toxicity accordingly. The ability to assess the possible health effects and risk of metal contamination to standard test species of fish, such as rainbow trout (*Oncorhynchus mykiss*), is a powerful tool in protecting aquatic systems.

Some degree of redundancy in the presentation of this PhD thesis was unavoidable because each chapter was written independently for publication in peer-reviewed journals. At the time of submission of this PhD thesis, Chapters 2, 3 and 4 had been previously published in the academic journals *Ecotoxicology*, *Environmental Science and Pollution Research*, and *Ecotoxicology and Environmental Safety*, respectively. Chapters 5 and 6 are to be submitted for publication as two parallel articles.

1.3 Objectives

The primary objective of this project was to characterize sensitivity of early life stage white sturgeon to metals of particular concern in aquatic systems, including Cu, Cd, Zn, and Pb, and to investigate the risk of exposure to metals in the UCR on early life stage sturgeon. To date, few studies have been conducted to characterize potential effects of metals on sturgeon. Previous work investigated the effects of Teck effluent and chronic exposure of Cu, Cd, and Zn, on early life stages of white sturgeon (Vardy D.W, 2011 MSc Thesis; Vardy et al. 2011). Results

indicated that under chronic conditions, early life stages of white sturgeon are relatively sensitive to the metals tested in comparison to other fishes, and that threshold values approach water quality criteria for certain metals such as copper, raising questions about the overall protectiveness of water quality standards. Results of studies of the Teck effluent suggested that exposure to dilutions of 25% effluent or less would not likely result in decreased survival or growth of early life stage white sturgeon. The next step was to further characterize sensitivity of white sturgeon to metals, focusing on acute exposures and characterizing sensitive life stages, especially to metals of particular interest such as copper. In addition, investigations into the effects of metals in the water column and sediment of the UCR on early life stage white sturgeon were needed.

In the present project, a combination of *in situ* (field) and controlled laboratory studies were conducted to assess effects of metals and selected environmental matrices, including Columbia River water and sediments, to white sturgeon. The studies and results presented in this dissertation investigated effects of:

1) Acute exposure to individual metals of concern, including Cu, Cd, Zn, and Pb, on early life stages of white sturgeon

2) Samples of water from the UCR on early life stages of white sturgeon

3) Samples of sediments from the UCR on early life stages of white sturgeon

Chapters 5 and 6 were conducted as part of a RI/FS under the oversight of the US EPA (www.ucr-rifs.com), and data obtained from this work will be used to supplement information in

a baseline ecological risk assessment (BERA) and overall RI/FS. In addition, data from the present project were used with the BLM to investigate and predict sturgeon sensitivity to metals of concern in the field, and to normalize data with varying ambient water quality parameters.

CHAPTER 2

Previous work indicated that under chronic exposure conditions, early life stages of white sturgeon are particularly sensitive to copper (Vardy et al. 2011). Therefore, characterizing the sensitivity of white sturgeon to copper is of particular interest. Concentrations of certain metals such as copper have been found to be elevated in sediments and other environmental matrices within the Columbia River (Bortleson et al. 2001; Cox et al. 2005; Majewski et al. 2003; Paulson et al. 2006). In addition, calculated thresholds for effects approached water quality criteria, raising questions as to whether sturgeon are being fully protected by current guidelines. Furthermore, water quality standards are typically derived from acute exposures with sensitive standard test organisms during sensitive life stages. Therefore, it is of interest to further characterize white sturgeon sensitivity to copper, at various life stages, and in comparison to other standard test fishes, to assess their level of risk. The following article has been previously published: Vardy D, Oellers J, Doering J, Hollert H, Giesy J, Hecker M. 2013. *Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper.* Ecotoxicology. 22: 139-147. A description of co-authorship can be found in Appendix F.

2.0 SENSITIVITY OF EARLY LIFE STAGES OF WHITE STURGEON, RAINBOW TROUT, AND FATHEAD MINNOW TO COPPER

2.1 Abstract

Populations of white sturgeon (*Acipenser transmontanus*) are in decline in several parts of the United States and Canada, attributed primarily to poor recruitment caused by degradation of habitats, including pollution with contaminants such as metals. Little is known about

sensitivity of white sturgeon to contaminants or metals such as copper (Cu). Here, acute (96 h) mortalities of white sturgeon early life stages due to exposure to Cu under laboratory conditions are reported. Two standard test species, rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*), were exposed in parallel to determine relative sensitivity among species. Swim-up larvae (15 days post hatch [dph]) and early juveniles (40-45 dph) of white sturgeon were more sensitive to Cu (LC50 = 10 µg/L and 9 - 17 µg/L, respectively) than were yolksac larvae (8 dph; LC50 = 22 µg/L) and the later juvenile life stage (100 dph; LC50 = 54 µg/L). White sturgeon were more sensitive of Cu than rainbow trout and fathead minnow at all comparable life stages tested. Yolksac larvae of rainbow trout and fathead minnow were 1.8 and 4.6 times, respectively, more tolerant than white sturgeon, while swim-up and juvenile life stages of rainbow trout were between 1.4- and 2.4- times more tolerant than white sturgeon. When plotted in a species sensitivity distribution with other fishes, the mean acute toxicity value for early life stage white sturgeon was ranked between the 1st and 2nd centile. The white sturgeon life stage of greatest Cu sensitivity coincides with the beginning of active feeding and close association with sediment, possibly increasing risk. White sturgeon early life stages are sensitive to aqueous copper exposure and site-specific water quality guidelines and criteria should be evaluated closely to ensure adequate protection.

2.2 Introduction

Sturgeon (Acipenseridae) are among the largest freshwater fish in the world. Some species can live more than one hundred years, weigh more than 800 kg and reach lengths of more than 6 meters. Sturgeon are also among the most archaic fish species with prehistoric ancestors

dating back an estimated 175 million years (UCWSRI 2002). Presently, however, populations of sturgeon are threatened globally and have been decreasing over the past century in Northern Europe, Asia, and North America (Birstein 1993; Coutant 2004; Gisbert and Williot 2002). In North America, populations of white sturgeon (*Acipenser transmontanus*) have been reported to be declining in the northwestern United States and British Columbia, Canada. Populations of white sturgeon have been listed as endangered in parts of Canada (COSEWIC 2013) and the USA (U.S. Fish and Wildlife Service 2013). Decreases in populations of sturgeon in the Columbia, Fraser, and Sacramento-San Joaquin rivers and their tributaries have been attributed primarily to poor annual recruitment (Coutant 2004; DFO 2007; Scott and Crossman 1998; UCWSRI 2002). Results of some simulation models of population trends and demographics have predicted that without implementation of successful remedial efforts the white sturgeon will become virtually extinct in these rivers within fifty years (DFO 2007; Irvine et al. 2007; Paragamian et al. 2005; Paragamian and Hansen 2008; UCWSRI 2002).

Possible hypotheses for failures of recruitment of white sturgeon include, among others, overharvesting, habitat alteration, changes in flow regime, decreased water quality, such as temperature, turbidity, total dissolved gases and pollution, poor nutrition, genetic bottlenecks or inbreeding depression, predation by introduced species such as walleye (*Sander vitreus*), inter-specific competition, pathogens, and disease (Birstein 1993; Coutant 2004; Gisbert and Williot 2002; Irvine et al. 2007; Kruse and Scarnecchia 2002a; Luk'yanenko et al. 1999; Paragamian and Hansen 2008; UCWSRI 2002). In some of the larger North American rivers, such as the Columbia, metals are of particular concern due to past and present activities of mines, metallurgical facilities, pulp and paper mills, as well as other industrial and municipal sources

(UCWSRI 2002). Copper (Cu), for example, is often found in contaminated systems at concentrations that are greater than naturally occurring levels (Grosell 2012; Kamunde and Wood 2003; Niyogi and Wood 2003). Concentrations of Cu in clean natural freshwaters are typically in the lower μg/L range (e.g. 0.2-2 μg/L), but thresholds for lethality on fishes can occur at concentrations that are only 10-fold greater (Grosell 2012; Wood 2001). In addition, effects on more sensitive endpoints, including behaviour, chemosensory, and olfaction, have been recorded within the lower concentration range (Grosell 2012). In general, little is known about the potential toxicity of metals, such as Cu, to white sturgeon or the tolerance of white sturgeon relative to other fishes.

Water quality guidelines and criteria are typically based upon effects concentrations (e.g. LC50's) for aquatic organisms and are estimates of the concentration of a contaminant in the environment that is expected to protect 95% of a group of diverse genera, assuming an appropriate number and variety of taxa are used for calculations (CCME 2007, EPA 1985). In cases where a species is deemed commercially or recreationally important and its threshold value is more sensitive than the calculated guideline or criteria, that particular species mean acute value (SMAV) will supersede (EPA 1985). These estimates, however, are only based upon species for which there are existing toxicity data that meet acceptable standards and often do not consider life stage specific sensitivities unless existing data indicates significant differences. Consequently, there is uncertainty whether an endangered species such as the white sturgeon that is recreationally, commercially, and culturally important (UCWSRI 2002) but has little to no existing toxicity data is protected by current guidelines and criteria.

Fish are generally most sensitive to effects of contaminants such as metals during early life stages (Hutchinson et al. 1998; McKim 1977). Previous work has indicated differences in sensitivity among early life stages of white sturgeon (Vardy et al. 2011), and studies of the effects of contaminants and life stage-specific sensitivities are important for making informed regulatory decisions. Life stage-specific sensitivity of white sturgeon is of particular interest given their early life history strategies. Early life stages of sturgeon inhabit benthic habitats, on surface sediments or in interstitial space between stones. There is some debate among researchers over the exact timing and sequence of certain behavioural events during white sturgeon early life stage development, these events possibly being influenced by differences in availability of appropriate substrata (McAdam 2011), but there is a general acceptance that early life stages of white sturgeon are in close contact with the substratum and exhibit distinct hiding and drifting phases (Brandon et al. 1983, 1985; Deng et al. 2002; Kynard and Parker 2005). Yolksac stage white sturgeon tend to hide/burrow in refugia (Brannon et al. 1983, 1985; Gessner et al. 2009; McAdam 2011; Richmond and Kynard 1995; personal observation in the laboratory). Prior to transitioning to exogenous feeding, sturgeon swim up in the water column (presumably to be transported by currents to more suitable foraging grounds; Auer and Baker 2002; Gessner et al. 2009; McAdam 2011) before returning to the bottom during the juvenile life stage, where they begin to scavenge and prey on benthic species and spend much of their life closely associated with sediments. Therefore, in addition to exposure to pollutants in the water column, sturgeon can be exposed to contaminants associated with sediments (Feist et al. 2005; Kruse and Scarnecchia 2002a) or contaminants released into the sediment-water interface. Sediments are sinks for pollutants and often contain elevated concentrations of metals, which can be released

back into pore water and the water column following remobilization (Salomons et al. 1987; Sullivan and Taylor 2003). Thus, white sturgeon could be exposed chronically to lesser concentrations of metals, or, during certain life stages and for shorter periods of time, to greater concentrations of metals at the sediment-water interface. For this reason, and to generate data to develop species sensitivity distributions (SSDs) in support of deriving protective acute water quality guidelines and criteria, it is necessary to determine both acute and chronic toxicity of metals to white sturgeon. The results of chronic studies on survival and growth have been presented previously (Vardy et al. 2011).

The primary objective of this study was to establish acute toxicity data for the effect of Cu on early life stages of white sturgeon that can be used in risk assessments. Early life stage white sturgeon were exposed to increasing concentrations of dissolved Cu, bracketing environmentally relevant concentrations and those expected to be lethal. In addition, rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) were exposed to Cu in the laboratory, in parallel to white sturgeon, to provide paired information for use in species sensitivity comparisons.

2.3 Methods

2.3.1 Test Materials

Copper (II) sulfate pentahydrate (Chemical Abstracts Service (CAS) number 7758-99-8; purity 99.995 %) was obtained from Sigma-Aldrich (Oakville, ON, Canada) and was dissolved in laboratory reverse osmosis water.

2.3.2 Experimental Fish

Fertilized white sturgeon eggs were collected at the Kootenay Trout Hatchery, Fort Steele, BC, Canada, from a minimum of four breeding pairs of adult white sturgeon caught in the Columbia River near Waneta, Canada. Fertilized eggs were transported to the Aquatic Toxicology Research Facility (ATRF), University of Saskatchewan, Saskatoon, SK, Canada where the embryos were raised under standard culturing conditions (Conte et al. 1988) until the desired life stages were achieved. Eyed embryos of rainbow trout were obtained from the Trout Lodge (Summer, WA, USA) and incubated in McDonald-type hatching jars (Aquatic Ecosystems, Apopka, FL, USA) until hatch. Fathead minnows were obtained from Osage Catfisheries (Osage Beach, MO, USA) and several generations were produced to insure healthy progeny.

2.3.3 Exposure Methods

Acute (96 h) toxicity of Cu was determined in accordance with the methods described by the American Society for Testing and Materials (ASTM 2007), with minor modifications. The exposure design consisted of sets of laboratory-based 96 h static renewal tests with mortality as the measurement endpoint. Laboratory water (carbon and bio-filtered city water) was adjusted to simulate natural conditions of the Columbia River near Trail, BC, Canada. Target hardness of ~65 mg/L and dissolved organic carbon (DOC) concentrations of ~2.5 mg/L were achieved by mixing laboratory water with reverse osmosis water in a 1:1 ratio. Target temperatures of 12 ± 1, 16 ± 1 and 20 ± 1 °C for rainbow trout, white sturgeon, and fathead minnows, respectively, were achieved by immersing the exposure chambers in chilled or heated water baths or by use of

environmental control chambers. All fish were tested under a 16:8 h light:dark cycle of illumination by use of standard daylight fluorescent lighting. Culture conditions for the fish (DOC, hardness, temperature, photoperiod) were the same as exposure conditions with the difference that fish were fed between one to four times a day, depending on life stage. For testing the yolksac life stage (8 dph) fish were exposed to increasing concentrations of Cu in 0.5 L high-density polyethylene (HDPE) test containers. Identical but larger 5L and 20L test containers were used during the older life stage toxicity tests. Loading densities remained less than the recommended 0.5 g/L and fish were not fed during the acclimation and exposure period (ASTM 2007). The various life stages, expressed as dph, and species tested are described in Table 2.1.

Table 2.1. Acute median lethal concentrations (LC50s) for copper exposure for white sturgeon (*Acipenser transmontanus*), rainbow trout (*Oncorhynchus mykiss*), and fathead minnow (*Pimephales promelas*) early life stages expressed in days post hatch (dph).

96hr LC50 for Copper Exposure (µg/L)								
Fish Species	Life stage						Water Quality Criteria	
	Yolksac (8 dph)	Swim-up (15 dph)	Juvenile (40 dph)	Juvenile (45 dph)	Later Juvenile (100 dph)	SMAV[a]	CMC[b]	CWQG[c]
White sturgeon (*Acipenser transmontanus*)	22 (20 – 25)	10 (8 – 12)	9 (7 – 12)	17 (14 – 21)	54 (47 – 62)	18	7.9 (± 1.5)	2
Rainbow trout (*Oncorhynchus mykiss*)	40 (34 – 46)	21 (18 – 23)	22 (20 – 25)	24 (20 – 28)		26	8.5 (± 3.0)	2
Fathead minnows (*Pimephales promelas*)	102 (78 – 135)					102	11.8	2

Values in brackets for LCs represent 95% confidence intervals; values in brackets for water quality criteria represent standard deviation.

[a] SMAV refers to the species mean acute value

[b] CMC refers to the Criteria Maximum Concentration for fresh water species. The mean freshwater criteria is presented and calculated from the various life stage experiments for each specie using the Biotic Ligand Model (EPA 2007b)

[c] CWQG refers to the Canadian Water Quality Guidelines for the protection of aquatic life adjusted to the present study's hardness (CCME 2003)

Concentrated stock solutions of Cu were prepared separately in individual HDPE carboys and allowed to equilibrate for 48 h prior to making dilutions to obtain test solutions. Exposures were conducted in triplicate or quadruplicate for each treatment group; each test chamber contained 10-15 individuals with 50% solution renewal every 12 h. Fish were acclimatized to the exposure chambers for 24 h prior to the addition of test solutions. Exposure chambers were cleaned once a day and dead fish were removed, length and weight measured, and preserved for potential use in future experiments.

2.3.4 Water Chemistry Analysis

Basic water quality variables, including temperature, pH, dissolved oxygen and conductivity, were measured daily by use of Symphony Electrodes (VWR, Mississauga, ON, Canada, Cat no. 11388-328) or YSR electrodes (YSR Inc., Yellow Springs, OH, USA). Typically, subsamples were collected during water changes from each replicate of each concentration and used for individual analysis. Hardness, alkalinity, ammonia, nitrates, nitrites, and chlorine were collected following a similar sampling scheme but only at the initiation and termination of experiments, and analyzed by use of LaMotte colorimetric and titrator test kits (Chestertown, MD, USA) or samples were sent to Columbia Analytical Services (CAS; Kelso, WA, USA) for external analyses. Water samples for analysis of concentrations of Cu in exposure chambers were also collected following the same sampling scheme at initiation and termination of the experiment. Water for Cu analysis was collected from each treatment group into acid-cleaned, polyethylene bottles and filtered through a 0.45 µm polycarbonate filter. Filtered water was acidified with ultrapure nitric acid to pH <2. Quantification of Cu was performed by use of

inductively coupled plasma mass spectrometry (ICP-MS) following EPA method 6020 and ILM05.2D (Creed et al. 1994). All calculations and reported values pertaining to Cu concentrations are based on the average measured concentrations in the treatment groups. DOC analysis was performed using a Total Organic Carbon (TOC) analyzer (TOC-5050A, Shimadzu, Mandel Scientific, Guelph, ON, Canada).

2.3.5 Data Analysis and Statistics

Mortality was calculated and the proportion of fish dead in each of the exposure chambers of a given Cu concentration was compared to that of the controls. LC50s for each of the species were calculated by use of TOXSTAT® software (Western EcoSystems Technology 1996). To assess the relative sensitivity of early life stages of white sturgeon to Cu, relative to those of other fishes, a species sensitivity distribution (SSD) was calculated for Cu (Posthuma et al. 2002). The SSD for freshwater fishes was derived based on toxicity data obtained from: EPA's ECOTOX database (EPA 2007a), information on Cu sensitivity of three different sturgeon species published by Dwyer et al. (2005), and the data obtained during this study. Data considered for the derivation of the SSD were exclusively from 96 h toxicity studies that reported LC50 values. Species mean (geomean) acute values (SMAVs) were calculated where data from multiple studies were available. If only one data point was available for a species, this was used as the SMAV in the SSD. To facilitate comparisons among tests without confounding the comparison by differences in hardness, all data included in the SSD were adjusted to a hardness of 50 mg $CaCO_3$/L by use of the Criteria Maximum Concentration (CMC) regression

equation for Cu, as outlined by the US EPA for calculating freshwater dissolved metals criteria that are hardness dependent (EPA 2009).

2.4 Results

2.4.1 Exposure Verification

Measured concentrations of Cu (Table 2.2) were comparable to nominal concentrations, and on average, were within 95 % of each other (see Appendix A, Supplemental Materials Table A1). However, there were small detectible concentrations of Cu in the control groups, but these concentrations were less than the least dose of each metal concentration. Generally, measured concentrations were less than nominal concentrations.

Table 2.2. Mean ± standard deviation (SD; numbers in brackets) measured exposure concentrations for copper during acute (96 h) static renewal exposure experiments with white sturgeon (WS; *Acipenser transmontanus*), rainbow trout (RT; *Oncorhynchus mykiss*), and fathead minnow (FM; *Pimephales promelas*) early life stages expressed as days post hatch (dph).

	Fish species									
Treatment	WS life stages (dph)					RT life stages (dph)				FM life stages (dph)
	8	15	40	45	100	8	15	40	45	8
	Measured (µg/L)	Measured (µg/L)	Measured (µg/L)	Measured (µg/L)	Measured (µg/L)	Measured (µg/L)	Measured (µg/L)	Measured (µg/L)	Measured (µg/L)	Measured (µg/L)
Control	1.3 (± 0.6)	0.4 (± 0.0)	0.4 (± 0.0)	0.4 (± 0.0)	0.8 (± 0.2)	1.3 (± 0.7)	0.3 (± 0.1)	2.4 (± 2.5)	1.2 (± 0.6)	1.4 (± 0.8)
1	1.8 (± 0.2)	2.8 (± 0.1)	1.6 (± 0.1)	2.0 (± 0.1)	8.3 (± 0.1)	1.8 (± 0..6)	2.1 (± 0.1)	9.7 (± 0.7)	2.6 (± 0.1)	2.1 (± 0.5)
2	2.7 (± N/A)*	4.7 (± 0.2	3.8 (± 0.2)	3.7 (± 0.1)	17 (± N/A)*	3.5 (± 0.6)	3.8 (± 0.0)	20 (± 1.4)	4.0 (± 0.0)	3.6 (± 0.7)
3	5.9 (± 0.1)	8.3 (± 0.1)	11 (± 0.4)	7.3 (± 0.1)	36 (± N/A)*	8.0 (± 0.7)	7.6 (± 0.0)	40 (± 2.2)	8.4 (± 0.3)	8.6 (± 0.7)
4	8.2 (± N/A)*	20 (± 0.9)	21 (± 1.3)	15 (± 0.2)	74 (± 21.6)	19 (± 3.6)	15 (± 0.2)	74 (± 8.5)	15 (± 0.4)	23 (± 1.9)
5	21 (± 1.8)	30 (± 0.1)	42 (± 1.7)	29 (± 0.1)	150 (± N/A)*	38 (± 2.4)	29 (± 0.0)	146 (± 14.6)	30 (± 0.5)	43 (± 1.0)
6	38 (± 0.2)	52 (± 3.2)	85 (± 2.1)	56 (± 0.4)	282 (± 24.4)	78 (± 11.5)	59 (± 0.5)	296 (± 18.7)	63 (± 2.4)	85 (± 12.4)
7	86 (± 10.7)		171 (± 2.1)			155 (± 32.3)				195 (± 9.3)
8	151 (± 0.4)									
9	382 (± 5.2)									

* Standard deviation was not calculated due to lack of concentration measurements.

2.4.2 Water Quality

Average water temperatures for all treatment groups during the white sturgeon, rainbow trout, and fathead minnow exposures were 16 °C (± 0.9), 13 °C (± 0.5), and 22 °C (± 0.1), respectively. The average dissolved oxygen saturation, pH, and conductivity for all treatment groups were 86 % (± 8.7), 7.5 (± 0.2), and 187 µS/cm (± 25.5), respectively. Mean hardness was 57 mg/L $CaCO_3$ (± 12.4) and the concentration of dissolved organic carbon was 2.2 mg/L (± 0.5). The average total concentration of ammonia, expressed as nitrogen (N) for all treatment groups was less than the limit of detection (< 0.025 mg/L). There were no significant differences in all other measured water quality parameters among treatment groups of any given experiment (summary of analytical methods, method detection limits, method blanks, and mean water quality parameters for individual exposures are provided in Appendix A, Supplemental Material Table A1-A3).

2.4.3 Lethal Concentrations

LC50s were successfully calculated for all life stages of each species that was tested (Table 2.1). Average survival of unexposed control fish was 90% or greater in all experiments (see supplementary data for summary of mean mortality for individual exposures). White sturgeon were most sensitive to Cu at 15 and 40 dph, followed by 45 and then 8 dph. White sturgeon exposed to Cu at a later life stage (100 dph) were more tolerant than were earlier life stages (Figure 2.1). LC50s for toxicity of Cu to white sturgeon were less than those for rainbow trout and fathead minnows for all comparable life stages tested (Figure 2.1, Table 2.1). LC50s for white sturgeon swim-up larvae and juvenile life stages were between 1.4- and 2.1- times

more sensitive than rainbow trout. Rainbow trout were least sensitive at 8 dph, followed by the later life stages, which exhibited comparable sensitivities to each other. Fathead minnow (8 dph) were more tolerant than white sturgeon and rainbow trout at all life stages tested.

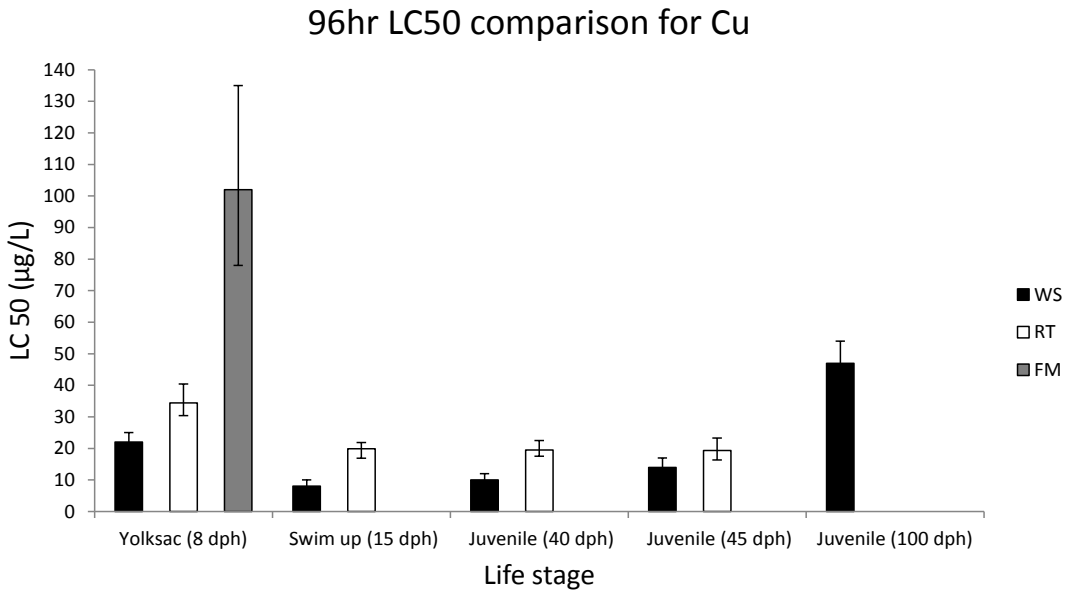

Figure 2.1. Median lethal concentrations (LC50s) comparison for copper exposure to white sturgeon (WS; *Acipenser transmontanus*), rainbow trout (RT; *Oncorhynchus mykiss*), and fathead minnow (FM; *Pimephales promelas*) exposures at various life stages (days post hatch [dph]).

Error bars represent confidence intervals for measured LC50s.

2.4.4 Species Sensitivity Distribution

The SSD that was developed based on 59 freshwater fishes, including the data of the various life stages of the three species studied here (Figure 2.2), demonstrated that white sturgeon that were 8, 15, 40, 45, or 100 dph were ranked in the 14th, 1st, 2nd, 3rd, and 28th centile, respectively. The SMAV for whites sturgeon ranked in the 2nd centile. Rainbow trout from the present study at 8, 15, 40, and 45 dph ranked in the 22nd, 13th, 9th, and 10th centile, respectively.

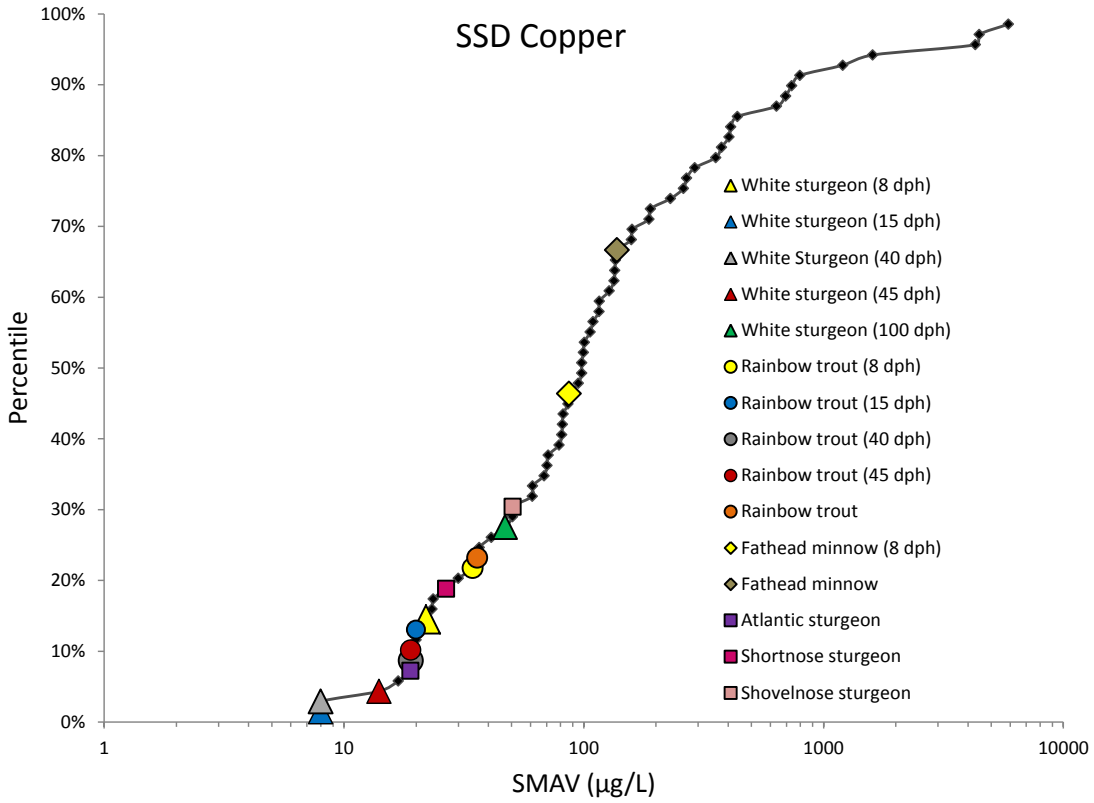

Figure 2.2. Fish species sensitivity distribution (SSDs) for copper.

Values for species with life stages, expressed as days post hatch (dph), are from experiments conducted at the University of Saskatchewan. Atlantic, shortnose, and shovelnose sturgeon values are from Dwyer et al. (2005), and all other species values are from the ECOTOX database (EPA 2007a). The Species Mean Acute Value (SMAV) is the geometrical mean LC50 for a given species.

The SMAV for rainbow trout calculated solely from the present study, when averaged among all life stages, ranked in the 8th centile, the SMAV for rainbow trout calculated solely from the ECOTOX database ranked in the 23rd centile, and the overall SMAV for rainbow trout, calculated from the ECOTOX database and values generated during the present study, ranked in the 16th centile. Based on the results of the present study, fathead minnow (8 dph) ranked in the 46th centile, while the SMAV for fathead minnow, calculated from the ECOTOX database,

ranked in the 67th centile. The overall SMAV for fathead minnow, calculated from the ECOTOX database and the present study's findings, ranked in the 59th centile. Early life stage Atlantic (*Acipenser oxyrinchus*), shortnose (*Acipenser brevirostrum*), and shovelnose (*Scaphirhynchus platorynchus*) sturgeon based on data from Dwyer et al. (2005) ranked in the 7th, 19th, and 30th centile, respectively.

2.5 Discussion

Based on findings of the present study, early life stage white sturgeon appear to be among the most sensitive fishes to acute Cu exposure, relative to other freshwater fishes. Three of the five life stages tested for white sturgeon were the most sensitive fishes in the SSD. The SMAV for white sturgeon was calculated and plotted in the SSD and white sturgeon were ranked the most sensitive species overall. Similarly, all other early life stage sturgeon incorporated in the same SSD, including Atlantic, shortnose, and shovelnose sturgeon, were relatively sensitive and ranked in the 23rd centile or less. Dwyer et al. (2005) concluded from their studies that sturgeon in general should be considered a sensitive species in contaminant assessments, and results from the present study are consistent with these findings. Results of previous studies have shown that some standard test species, such as rainbow trout, are relatively sensitive to certain metals, whereas others, such as fathead minnow, are more tolerant (Besser et al. 2007; Dwyer et al. 2005; Taylor et al. 2000). LC values for the effects of Cu on rainbow trout and fathead minnow determined during the present study were slightly less, but generally consistent with previously reported SMAVs.

Post hatch, early life stages of fish are generally considered more sensitive to contaminant exposure than adults (Hutchinson et al. 1998; McKim 1977, 1978). In the present study, five early life stages of white sturgeon and four early life stages of rainbow trout were exposed to Cu to compare life stage specific sensitivity. For both species, the late larval/early juvenile life stages (15 – 45 dph) were more sensitive to the effects of Cu than was the yolksac (8 dph) life stage, and in the case of white sturgeon, the later juvenile life stage (100 dph). Greater sensitivity to Cu following the initial yolksac life stage and greater tolerance during the later juvenile life stage was observed for white sturgeon (Figure 2.1). The observed differences in tolerance might be due to the fact that 8 dph larvae are still absorbing their yolksacs whereas at 15 dph larvae have begun to switch to exogenous feeding and are more physically active, leading to greater exposure since rates of respiration are increased and more water is forced over the gills. Rainbow trout, however, did not display a similar trend in sensitivity to Cu following 8 dph that was observed in white sturgeon. This might be due to differences in duration and timing of development of rainbow trout and white sturgeon, such that the observed sensitivities to Cu among the time periods (dph) tested might not be entirely comparable between species. Under culture conditions, rainbow trout embryos are typically incubated much longer than white sturgeon (4-14 weeks, depending on water temperatures, compared to one week for white sturgeon), and absorption of the yolksac can occur over a period as twice as long. Therefore, longer development might result in less of a difference in sensitivity to Cu of post-yolksac larvae because rainbow trout are not transitioning through similar developmental stages as white sturgeon at comparable ages and at the same speed.

Significant differences in sensitivities among early life stages could have major implications in risk assessment and development of water quality guidelines and criteria. Risk assessments based on the assumption that younger fish tend to be more sensitive to contaminants than older fish could result in considerable underestimations of sensitivity if post yolksac early life stages are not considered, as demonstrated in the present study, and lead to under-protection of certain species. Currently, in Canada and the United States, there is no requirement to evaluate differences in sensitivities among life stages when developing water quality guidelines and criteria. In the United States, differences in life stage sensitivity are taken into account but only if existing data demonstrates that there are differences of more than a factor of two (EPA 1985). If no toxicity data exist for various life stages of a species, then differences in sensitivities among life stages are not considered when calculating criteria or assessing potential for effects. To overlook these potential differences with a vulnerable population of fish could result in significant under-protection. Yolksac larvae of white sturgeon have greater tolerance to Cu toxicity compared to succeeding life stages, but since these earlier life stages might be in more intimate contact with biologically available metals in contaminated sediments, they may be more at risk than would be indicated solely by their tolerance to Cu concentrations. Greater exposure to Cu during the transition to exogenous feeding, however, is detrimental since previous studies have shown that white sturgeon are inherently sensitive during this period of development (Vardy et al. 2011). This poses an increased threat to early life stage juveniles that return to the bottom to feed and are at greater risk of exposure to sediment bound contaminants.

Early life stages of white sturgeon are sensitive to aqueous copper exposure and site-specific water quality guidelines and criteria should be evaluated closely to ensure adequate

protection when sturgeon are of concern. The Canadian water quality guideline for the protection of aquatic life for Cu, adjusted to the present study's hardness of 57 mg/L $CaCO_3$, is 2 µg/L (CCME 2003). In the United States, criteria for protection of aquatic life for Cu are site-specific and freshwater criteria are calculated by use of the biotic ligand model (BLM; EPA 2007b). Based on the present study's water quality parameters for white sturgeon, the water quality acute criteria for Cu, recommended by the US EPA for protection of aquatic life (CMC; EPA 2007b, 2009), would be between 6.4 and 9.5 µg/L. In order to assess the degree of protection of white sturgeon in relation to water quality guidelines and criteria, one half the species mean acute value (½ SMAV) was calculated. This is similar, but on a species level, to EPA water quality criteria methods where one half final acute values (FAV's) are calculated. In the present study, ½ SMAV for white sturgeon is above the Canadian water quality guideline but falls within the calculated range for US criteria (Table 2.1). When half the LC50 values for the individual life stages of white sturgeon are examined, some thresholds are less than US criteria. This merits further investigation, especially at the more sensitive life stages, to assess the level of protection in relation to water quality criteria for Cu.

This study provides a portion of much needed toxicity data for early life stage white sturgeon and identified significant differences in sensitivities among early life stages of fish. LC50 values from the present study predicted similar trends in early life stage white sturgeon sensitivity when compared to chronic early life stage white sturgeon threshold values for Cu (chronic values: 19 dph = 9.9 µg/L and 58 dph = 12.4 µg/L; Vardy et al. 2011). When feasible, contaminant exposure studies should include different life stages to help elucidate possible differences in life stage sensitivities in order to develop more comprehensive water quality

guidelines and criteria. White sturgeon are sensitive to Cu exposure and water quality guidelines and criteria may need to be evaluated on a site-by-site basis when white sturgeon early life stages are present in order to ensure protection. Other endpoints, such as effects of Cu exposure on olfaction, chemosensory, and/or behavior, for example, could also be investigated with white sturgeon as these have been shown to be the most sensitive endpoints in other fish species (Grosell 2012). In addition, alternate routes of exposure, such as from contaminated sediment or dietary uptake, warrant further investigation as water-only exposures may represent variable proportions of total exposure depending upon life stage.

CHAPTER 3

Previous work (Chapter 2 and Vardy et al. 2013) indicated that sturgeons in general, and white sturgeon specifically, are sensitive to copper during acute exposures compared to other fishes, especially during early life stages. This raised significant concerns regarding the potential risk to white sturgeon because of exposure to metals in surface waters such as the Columbia River. To further characterize sensitivity of white sturgeon to metals and application to conditions in the Upper Columbia River (UCR), acute exposures with cadmium, lead, zinc, and copper, were conducted in parallel in the laboratory with standard laboratory water and in the field with Columbia River water.

Physical and chemical properties of test solutions can affect the toxicity of metals to an organism. This can make assessment of field scenarios based on laboratory data difficult due to the uncertainties associated with the unique water quality characteristics at field sites. To address such uncertainties, a parallel assessment of the toxicity of a metal in field and laboratory water was conducted in order to derive a more accurate estimate of potential environmental issues related to the exposure of the test organism with the metal of concern. The purpose of this study was to establish laboratory and field acute toxicity data for the exposure of early life stages of white sturgeon to the metals of concern that can be used in UCR risk assessments, and to develop UCR water specific water effects ratios (WERs) for these four elements. WER's were performed to assess the relative bioavailability of these metals in UCR water compared to laboratory water, and to elucidate possible differences in the sensitivity of early life stage white sturgeon to the same concentrations of metals when tested in UCR water as compared to laboratory water. The

following article has been previously: Vardy D, Santore R, Ryan A, Giesy J, Hecker M. 2014 a. *Acute toxicity of copper, lead, cadmium, and zinc to early life stages of white sturgeon (Acipenser transmontanus) in laboratory and Columbia River water.* Environmental Science and Pollution Research. *In Press*. A description of co-authorship can be found in Appendix F.

3.0 ACUTE TOXICITY OF COPPER, LEAD, CADMIUM, AND ZINC TO EARLY LIFE STAGES OF WHITE STURGEON (*ACIPENSER TRANSMONTANUS*) IN LABORATORY AND COLUMBIA RIVER WATER

3.1 Abstract

Populations of white sturgeon (*Acipenser transmontanus*) are in decline in North America. This is attributed, primarily, to poor recruitment, and white sturgeon are listed as threatened or endangered in several parts of British Columbia, Canada, and the United States. In the Columbia River, effects of metals have been hypothesized as possible contributing factors. Previous work has demonstrated that early life stage white sturgeon are particularly sensitive to certain metals, and concerns over the level of protectiveness of water quality standards are justified. Here we report results from acute (96-h) toxicity tests for copper (Cu), cadmium (Cd), zinc (Zn), and lead (Pb) from parallel studies that were conducted in laboratory water and in the field with Columbia River water. Water effect ratios (WERs) and sensitivity parameters (i.e. median lethal accumulations, or LA50s) were calculated to assess relative bioavailability of these metals in Columbia River water compared to laboratory water, and to elucidate possible differences in sensitivity of early life stage white sturgeon to the same concentrations of metals when tested in the different water sources. For Cu and Pb, white sturgeon toxicity tests were initiated at two life stages, 8 and 40 days post hatch (dph), and median lethal concentrations

(LC50s) ranged between 9-25 µg Cu/L and 177-1556 µg Pb/L. LC50s for 8 dph white sturgeon exposed to Cd in laboratory water and river water were 14.5 µg/L and 72 µg/L, respectively. Exposure of 8 dph white sturgeon to Zn in laboratory and river water resulted in LC50s of 150 µg/L and 625 µg/L, respectively. Threshold concentrations were consistently less in laboratory water compared to river water, and as a result, WERs were greater than 1 in all cases. In addition, LA50s were consistently greater in river water exposures compared to laboratory exposures in all paired tests. These results, in combination with results from the Biotic Ligand Model (BLM), suggest that the observed differences in toxicity between river water exposures and laboratory water exposures are not entirely due to differences in water quality and metal bioavailability, but rather in combination with differences in fish sensitivity. It is hypothesized that differences in concentrations of calcium in the different water sources might have resulted in differences in acquired sensitivity of sturgeon to metals. Canadian water quality guidelines, US national criteria for the protection of aquatic life, and water quality criteria for the state of Washington were less than LC50 values for all metals and life stages tested in laboratory and Columbia River water. With the exception, however, that 40 dph white sturgeon exposed to Cu in laboratory water resulted in threshold values that bordered US national criteria and criteria for the state of Washington.

3.2 Introduction

Sturgeons and paddlefishes (Acipenseroidei) are threatened throughout the world and many species are at risk of extinction due to anthropogenic impacts on the environment. Over-harvesting, loss and alteration of habitat, and pollution are common factors that have contributed

to decreases in abundances of populations (Birstein 1993; Gisbert and Williot 2002; Hu et al. 2009; Irvine et al. 2007; Luk'yanenko et al. 1999; Paragamian and Hansen 2008). As of 2010, the International Union for Conservation of Nature (IUCN) listed sturgeons as the most critically endangered fish species, with over 90% of sturgeons classified as either near threatened, vulnerable, endangered, or critically endangered (Jaric 2012; IUCN 2011). Despite the global decline of sturgeons and paddlefishes and their endangered status, there has been relatively little research into the effects of pollution on this family of fish in comparison to taxa that are more commonly studied in ecotoxicology. Sturgeons inhabit large, industrialized river systems that are often more severely polluted than smaller streams. Furthermore, previous studies have found sturgeons to be relatively sensitive to pollutants in comparison to other fishes, further emphasizing the need for more research into this issue (Dwyer et al. 2005).

In North America, the white sturgeon, *Acipenser transmontanus*, is the largest freshwater fish and exhibits great longevity. In the last half century many populations of white sturgeon have been experiencing annual recruitment failures. Reasons for these failures are not fully understood but results of some simulation models of trends and demographics of populations have predicted that without implementation of successful remedial efforts white sturgeon will become virtually extinct in some North American rivers within fifty years (DFO 2007; Irvine et al. 2007; Paragamian et al. 2005; Paragamian and Hansen 2008; UCWSRI 2002). Consequently, the white sturgeon has been listed as endangered in parts of Canada (COSEWIC 2013) and the USA (U.S. Fish and Wildlife Service 2013).

The population of white sturgeon in the Columbia River between Grand Coulee Dam in the USA and Hugh L. Keenleyside Dam in southern British Columbia, Canada, has been experiencing poor recruitment for over forty years (Hildebrand et al. 1999; Irvine et al. 2007). Information on this population was gathered between Hugh L. Keenleyside Dam and the U.S.-Canada border, and the number of individuals estimated to be 1,120 in 1995 and 1,157 individuals in 2004 (R.L. & L. Environmental Services 1996; Irvine et al. 2007). Based on an analysis of frequency distributions of lengths, it was speculated that this subpopulation has experienced recruitment that is less than that required to maintain a stable population and age distribution (Hildebrand et al. 1999; Irvine et al. 2007). There is evidence that adult white sturgeon are spawning and depositing viable eggs in certain areas of this reach in Canada, in particular at Waneta Eddy, which is located at the Pend'Oreille River-Columbia River confluence, just north of the U.S.-Canada border (Golder Associates Ltd. 2007, Howell and McLellan 2006). However, limited numbers of early life stage white sturgeon have been observed after they hatched in the river, and few naturally spawned juveniles have been observed in areas considered to be suitable habitats. However, juveniles (9–10 months old) that have been released into the Canadian section of the Columbia River as part of the White Sturgeon Recovery Plan exhibited good survival, growth rates, and body condition (Howell and McLellan 2006; UCWSRI 2002). A definitive cause for why this white sturgeon population is experiencing a life stage specific bottleneck is not yet known but as a result, in 2006, the population was classified as endangered by the government of Canada under the Canadian Species at Risk Act (SARA 2012).

Possible hypotheses for these failures of recruitment of white sturgeon include, among others, overharvesting, alteration of habitat, changes in flow regime, genetic bottlenecks or inbreeding depression, predation by introduced species such as walleye (*Sander vitreus*), inter-specific competition, pathogens, disease, and decreased water quality such as temperature, turbidity, total dissolved gases, pollution, and poor nutrition (Birstein 1993; Coutant 2004; Gisbert and Williot 2002; Irvine et al. 2007; Kruse and Scarnecchia 2002a; Luk'yanenko et al. 1999; Paragamian and Hansen 2008; UCWSRI 2002). White sturgeon inhabit large river systems such as the Columbia where contamination due to past and present activities of mines, metallurgical facilities, pulp and paper mills, as well as other industrial and municipal sources is often of concern (MacDonald et al. 1997; UCWSRI 2002). Little toxicity data exist, however, characterizing white sturgeon sensitivity to contaminants. This information is necessary to determine the relative sensitivities of white sturgeon for use in site-specific estimates of risk. Results of previous studies indicate that early life stages of white sturgeon are relatively sensitive to certain metals (Vardy et al. 2011, 2012). In addition, thresholds for effects of metals such as Cu to white sturgeon approach North American water quality criteria and guidelines (WQC and WQGs, respectively) for the protection of aquatic life, and there was concern whether white sturgeon were being fully protected by the current water quality standards (Vardy et al. 2012).

Difficulties arise, however, when trying to translate laboratory results to field conditions. Differences in environmental parameters, such as pH, hardness, and dissolved organic carbon (DOC) concentrations, can greatly affect the toxicity of a metal to an organism (Fairbrother et al. 2007). When developing site specific water quality standards for metals, threshold values are typically adjusted for hardness (CCME 2003; Stephan et al. 1985). In certain cases, toxicity tests

may be conducted in site specific water in order to develop water effect ratios (WERs) for greater specificity in developing standards. Logistically, however, this is not always feasible and can be costly. For certain metals, the biotic ligand model (BLM; Di Toro et al. 2001; Santore et al. 2001, 2002; HydroQual 2007), a metal speciation and predictive toxicity model, can be used to account for variability in water quality characteristics and normalize thresholds for effects. As of 2007, WQC for Cu in the United States are site specific and calculated by use of the BLM (EPA, 2007).

The primary objectives of this study were to evaluate acute lethality of early life stages of white sturgeon to Cu, Pb, Cd, and Zn, in both laboratory water and Columbia River water, and to assess the protectiveness of ambient WQC/WQGs to Columbia River white sturgeon. Early life stage white sturgeon were exposed to a range of concentrations of dissolved Cu, Cd, Zn, and Pb, bracketing environmentally relevant concentrations and those expected to be lethal. Acute exposures to metals were conducted in parallel in the laboratory and in the field with Columbia River water to assess the relative bioavailability of these metals in Columbia River water compared to laboratory water, and to elucidate possible differences in the sensitivity of early life stage white sturgeon to the same concentrations of metals when tested in the different water sources.

3.3 Methods

3.3.1 Test Materials

Copper (II) sulfate pentahydrate (Chemical Abstracts Service (CAS) number 7758-99-8; purity 99.995 %), cadmium chloride hemi-pentahydrate (CAS number 7790-78-5; purity

99.999%), zinc chloride (CAS number 7646-85-7; purity 98%) and lead nitrate (CAS number 10099-74-8; purity 99.99%) were obtained from Sigma-Aldrich (Oakville, ON, Canada). Stock solutions were prepared for all metals by dissolving chemicals in laboratory reverse osmosis water or Columbia River water collected upstream from the metallurgical facility in Trail, BC, Canada.

3.3.2 Experimental Fish

Fertilized white sturgeon eggs were collected at the Kootenay Trout Hatchery, Fort Steele, BC, Canada, from a minimum of four breeding pairs of adult white sturgeon caught in the Columbia River near Waneta, Canada. Fertilized eggs were transported to exposure facilities in Trail, BC, Canada and to the University of Saskatchewan, Saskatoon, SK, Canada, where the embryos were raised under standard culturing conditions (Conte et al. 1988) until the desired life stages were achieved.

3.3.3 Exposure Methods

Sets of laboratory- and field-based acute 96-h toxicity tests were conducted in parallel, under the same exposure conditions and following the same experimental protocols, at the Aquatic Toxicology Research Facility (ATRF), University of Saskatchewan, Saskatoon, Saskatchewan, and in retrofitted commercial trailers (Britco, Vancouver, BC, Canada) located adjacent to the Columbia River, upstream of the Teck Trail smelter facility (river mile 758: 49°07’01.32”N; 117°43’27.25”W), respectively. Exposures were initiated at 8 days post hatch (dph) and were conducted under static renewal conditions in decontaminated 0.5 L

polypropylene containers, per American Society for Testing and Materials (ASTM) guidelines for testing early life stage of fish (ASTM 2007, 2009). Exposures to Cu or Pb were also initiated at 40 dph and conducted under static renewal conditions, in larger 5 L decontaminated polypropylene containers. Fifty percent of the test solution was replaced every 12 hrs for each life stage tested. All exposures were conducted under a 16:8 h light:dark cycle of illumination by use of standard daylight fluorescent lighting, and with a target temperature of 16 ± 1 °C. Conditions for culturing white sturgeon, including dissolved organic carbon (DOC), hardness, temperature, and photoperiod, were the same as exposure conditions with the difference that fish were fed frozen bloodworms between one to four times a day, depending on life stage. Loading densities remained less than the recommended 0.5 g/L and fish were not fed during the acclimation and exposure period (ASTM 2007).

Stock solutions at the ATRF were prepared in reverse osmosis (RO) water and adjusted to a target water hardness of approximately 60 milligrams as calcium carbonate per liter (mg/L $CaCO_3$) and a target dissolved organic carbon (DOC) concentration of approximately 2 mg/L, to simulate natural conditions of the Columbia River near Trail, BC, Canada, by adding laboratory (i.e., dechlorinated City of Saskatoon water) in a 1:1 ratio. In the field, stock solutions were prepared by mixing chemicals directly with Columbia River water. All stock solutions were allowed to equilibrate for a minimum of 24 hrs prior to use. Exposure chambers were cleaned once a day and mortality and routine observations were recorded at time (t) = [0, 24, 48, 72, and 96] hrs. At the conclusion of the test, sturgeon were euthanized by use of tricaine methanesulfonate (MS 222), removed, and preserved. The dosing regimen of the different metals

in the laboratory and river water exposures at the various life stages, expressed as dph, are described in Table 3.1.

3.3.4 Water Chemistry

Routine water quality variables, including temperature, pH, DO, and conductivity, were recorded daily with Symphony Electrodes (VWR, Mississauga, ON, Canada, Cat no. 11388-328). Hardness, alkalinity, ammonia, nitrates, nitrites, and chlorine were measured at the initiation and termination of experiments by use of LaMotte colorimetric and titrator test kits (Chestertown, MD, USA). Concentrations of metals in exposure chambers were measured at initiation and termination of the experiments, except for the Cd and Zn river water tests. Equipment and personnel constraints during these experiments limited the water sampling capacities in the field. Therefore, only stock solutions and background concentrations of Cd and Zn in dilution water were assessed. The stock solution values were used to extrapolate actual concentrations in each treatment group by correcting for % recovery of metals in the stock solutions, and by adding the average background concentrations of metals in river water measured over the period of the exposure studies. Samples were collected using acid-cleaned high density polyethylene (HDPE) bottles, filtered through a 0.45 micrometer (μm) polyethersulfone membrane filter with Nalgene® filter holders and receivers; acidified with ultrapure nitric acid to a pH <2 standard units (s.u.), and maintained at approximately 4°C for shipment to the analytical laboratory (Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada, or Columbia Analytical Services, Kelso, WA, USA). All calculations and reported values pertaining to the dosing of metals in laboratory and river water are based on

Table 3.1. Nominal, and mean ± standard deviation (SD; numbers in brackets) measured exposure concentrations for copper, lead, cadmium, and zinc during 96 hour acute exposures in laboratory water (Lab) and Columbia River water for early life stages of white sturgeon (*Acipenser transmontanus*), expressed in days post hatch (dph).

Metal	Copper						Lead (µg/L)						Cadmium (µg/L)				Zinc (µg/L)			
Life stage	8 dph				40 dph		8 dph				40 dph		8 dph				8 dph			
Exposure Water	Lab		Columbia River		Lab		Columbia River		Lab		Columbia River		Lab		Columbia River		Lab		Columbia River	
	Nom. (µg/L)	Meas. (µg/L)	Nom. (µg/L)	Meas. (µg/L)	Nom. (µg/L)	Meas. (µg/L)	Nom. (µg/L)	Meas. (µg/L)	Nom. (µg/L)	Meas. (µg/L)	Nom. (µg/L)	Meas. (µg/L)	Nom. (µg/L)	Meas. (µg/L)	Nom. (µg/L)	Meas. (µg/L)	Nom. (µg/L)	Meas. (µg/L)	Nom. (µg/L)	Meas. (µg/L)
Control	0	1.3 (± 0.6)	0	0.8 (± 0.1)	0	0.4 (±0.0)	0	1.1 (± 0.1)	0	0.1 (±0.1)	0	0.2 (± 0.2)	0	0.1 (±0.1)	0	0.3 (± 0.3)	0	0.1 (±0.1)	0	16 [a]
1	2	1.8 (± 0.2)	2	1.6 (± 0.3)	1	1.6 (±0.1)	1	2.0 (± 0.2)	1	0.8 (±0.0)	1	0.4 (± 0.2)	31	21 (±0.2)	31	20 (± 3.9)	1.95	0.9 (±1.2)	19.5	34 [a]
2	3	2.7 [a]	3	4.1 (± 2.1)	4	3.8 (±0.2)	4	4.2 (± 0.2)	3	2.3 (±0.0)	3	1.4 (± 0.4)	61	46 (± 2.7)	61	37 (± 16)	7.81	7.6 (±0.7)	78.1	88 [a]
3	6	5.9 (± 0.1)	6	5.9 [a]	12	11 (±0.4)	12	10 (± 0.1)	9	6.4 (±0.7)	9	6.1 (± 2.0)	122	97 (±4.0)	122	95 (± 5.3)	31.25	26 (±2.6)	313	303 [a]
4	9	8.2 [a]	9	6.4 (± 0.2)	24	21 (±1.3)	24	20 (± 0.4)	27	19 [a]	27	17 (± 0.9)	244	208 (± 4.9)	244	192 (± 14)	125	107 (±8.9)	1250	1164 [a]
5	27	21 (± 1.8)	27	22 (± 1.6)	48	42 (±1.7)	48	42 (± 0.5)	81	65 (±3.2)	81	60 (± 2.3)	488	396 (± 11)	488	325 (± 117)	500	432 (±26)	5000	4606 [a]
6	54	38 (± 0.2)	54	46 (± 1.4)	96	85 (±2.1)	96	82 (± 0.7)	243	210 (±21)	243	191 (± 8.4)	976	809 (± 78)	976	799 (± 39)				
7	108	86 (± 10.7)	108	91 (± 6.3)	192	171 (±2.1)			486	414 (±23)	486	410 (± 49)	1952	1,610 [a]	1952	1685 (± 35)				
8	216	151 (± 0.4)	216	176 (± 13)																
9	400	382 (± 5.2)	400	327 [a]																

[a] Only one measurement value available

these measured concentrations. A summary of analytical methods and associated method detection limits, as well as characteristics of blanks, are provided in the Appendix B, Supplemental Materials Table B2.

3.3.5 Data Analysis and Statistics

Mortality was calculated and the proportion of fish dead in each of the exposure chambers of a given metal concentration was compared to that of the controls. All data for calculations of measured concentrations and mortality were expressed as the mean ± standard deviation (SD). When comparing treatments to control groups, or when applicable, blanks, data were tested for normality by use of the Shapiro-Wilk test or probability plots. When data were normally distributed or approximated normal distribution, analysis of variance (ANOVA) and a Dunnett's post hoc test were used to detect significant differences. In cases where data were not normally distributed, statistical analysis was conducted using the Kruskal Wallis test followed by the Mann–Whitney U test. Systat statistical software (Systat Software Inc., Chicago, IL, USA) was used for these analyses. Statistical significance was accepted when $p < 0.05$. Effective concentrations at which 50% mortality occurred (LC50s) were calculated by use of TOXSTAT® software (Western EcoSystems Technology 1996) and confidence intervals (CI) were presented in brackets. Experiments with 8 dph white sturgeon exposed to Cu were of increased interest and as a result additional toxicity tests were conducted with refined dosing regimens and increased water sampling. Following statistical comparisons, mortality results from the two experiments were combined to better estimate threshold values. To compare threshold values derived for white sturgeon exposed in laboratory water versus Columbia River water, WERs were calculated

following Equation 1. All laboratory water exposure data for WER calculations were adjusted to the corresponding hardness of river water by use of the Criteria Maximum Concentration (CMC) regression equations (EPA, 2009).

WER Columbia River = LC50 (field)/ LC50 (lab) (Equation 3.1)

Procedures for normalizing LC50 values to varying water chemistry parameters with the BLM followed methods outlined in the EPA Cu criteria document (EPA 2007b). In short, the BLM was first applied to the toxicity data (in speciation mode) and the sensitivity parameter (i.e. median lethal accumulation, or LA50) was determined for white sturgeon using the concentrations of metals (Table 3.1) and associated toxicological responses (Table 3.2) for Cu, Pb, Cd, and Zn effects to white sturgeon. The BLM can be used in toxicity mode to predict metal toxicity to a number of aquatic organisms for a range of ambient water quality characteristics. The purpose of first using the BLM in speciation mode is to determine the LA50s for white sturgeon that will allow the BLM to predict acute effect concentrations for white sturgeon exposed to Cu, Pb, Cd, and Zn under specified exposure conditions. This allows the BLM to translate effects from laboratory conditions to a wider variety of exposure scenarios where factors such as pH, DOC, hardness, and alkalinity may vary. To determine the LA50 for each metal, the BLM was run with measured water quality parameters, including temperature, pH, DOC, chloride (Cl^-), sulfate (SO_4^-), and alkalinity, or sometimes estimated water quality parameters, including calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and sodium (Na^+) for Cd and Zn river water experiments. Parameters that were not measured were estimated from the

average concentrations measured in water samples used in related exposures and studies (Tompsett et al. 2014).

Table 3.2. Mean ± standard deviation percent mortality for early life stages of white sturgeon (*Acipenser transmontanus*) after 96 hours of exposure to copper, lead, cadmium, and zinc and in laboratory water (Lab) and Columbia River water (CR).

Metal	Average Percent Mortality- Copper				Average Percent Mortality- Lead				Average Percent Mortality- Cadmium		Average Percent Mortality- Zinc	
Life stage	8 dph		40 dph		8 dph		40 dph		8 dph		8 dph	
Exposure Water	Lab	CR	Lab	CR	Lab	CR	Lab	CR	Lab	CR	Lab	CR
Control	0 (± 0)	0.8 (± 0.3)	6.7 (± 12)	0 (± 0)	1.6 (± 3.1)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	1.3 (± 2.8)	0 (± 0)	1.3 (± 2.8)
1	1 (± 2.7)	0 (± 0)	6.7 (± 12)	10 (± 0)	0 (± 0)	0 (± 0)	6.7 (± 12)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
2	0 (± 0)	4.8 (± 0.6)	0 (± 0)	3.3 (± 5.8)	0 (± 0)	0 (± 0)	0 (± 0)	6.7 (± 12)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
3	0 (± 0)	0 (± 0)	60 (± 27)	20 (± 20)	0 (± 0)	2.3 (± 4.5)	3.3 (± 5.8)	10 (± 17)	98 (± 0.6)	4.4 (± 3.8)	100 (± 0)	0 (± 0)
4	9.7 (± 8.1)	4.8 (± 0.6)	79 (± 20)	57 (± 21)	1.7 (± 3.3)	2.3 (± 4.5)	13 (± 15)	3.3 (± 5.8)	100 (± 0)	95 (± 8.2)	100 (± 0)	96 (± 3.7)
5	39 (± 29)	29 (± 2.6)	97 (± 5.8)	87 (± 5.8)	0 (± 0)	4.5 (± 5.2)	47 (± 5.8)	3.3 (± 5.8)	100 (± 0)	98 (± 3.8)	100 (± 0)	100 (± 0)
6	92 (± 10)	86 (± 2.2)	100 (± 0)	100 (± 0)	82 (± 11)	0 (± 0)	77 (± 15)	10 (± 10)				
7	97 (± 5.2)	99 (± 2.1)	100 (± 0)		100 (± 0)	2.3 (± 4.5)	63 (± 15)	67 (± 25)				
8	100 (± 0)	100 (± 1.4)										
9	100 (± 0)	100 (± 0)										

Concentration-response relationships between dissolved metal and observed mortality, normalized for bioavailability by considering accumulation on the biotic ligand (i.e. gill), were developed. From the concentration-response on the biotic ligand, the LA50s can be estimated. Site specific water quality parameters can then be used in combination with the white sturgeon BLM parameter file and the calculated LA50 to normalize concentration-response relationships or predict thresholds for effects (i.e. LC50s) in the field.

3.4 Results

3.4.1 Exposure Verification

Mean measured concentrations were generally in good agreement with nominal concentrations, with a slight underestimation of the targeted concentration in all treatments, except in the control groups and the lowest doses of Zn, which had slight overestimations (Table

3.1). Concentrations within exposure chambers remained consistent throughout the period of exposure. Small yet statistically significant concentrations of Pb were observed in the blanks. As a result, there is a degree of uncertainty associated with a limited number of Pb concentrations recorded. However, concentrations of Pb in blanks were always in the sub-microgram/litre range. Since no effects were observed at concentrations less than 243 µg Pb/L, this amount of Pb had no significant effect on estimates of thresholds for mortality.

3.4.2 Water Quality

Water quality measurements recorded throughout the duration of the study, including major cations and anions used in BLM calculations, remained relatively constant throughout the exposures (Table 3.3 and Appendix B, Supplemental Materials Table B1*)*. Overall, mean water temperatures for all treatment groups during exposures in laboratory water were 16 °C (± 0.9). Mean dissolved oxygen saturation, pH, and conductivity for all treatment groups in laboratory water were 86 % (± 8.7), 7.5 (± 0.2), and 187 µS/cm (± 25.5), respectively. Total concentrations of ammonia nitrogen for all treatments in river water were less than 0.025 mg/L. Mean DOC in laboratory water and river water was 2.3 mg/L (± 0.4) and 2.0 mg/L (± 0.2), respectively. However, there was a small amount of contamination during some of the sampling events, possibly due to the type of filters that were used. A polyethersulfone membrane filter was used to allow for a greater variety of possible analyses, such as metals and DOC, but might have contributed to increased DOC levels. As a result, DOC values were adjusted for contamination prior to being used in calculations, either by subtracting blank measurements or by use of total

Table 3.3. Acute median lethal concentrations (LC50s) of early life stages of white sturgeon (*Acipenser transmontanus*), expressed in days post hatch (dph), exposed to copper, lead, cadmium, and zinc in laboratory water (Lab.) and Columbia River water (C.R.).

Metal	Copper (µg/L)				Lead (µg/L)				Cadmium (µg/L)		Zinc (µg/L)	
Life stage	8 dph		40 dph		8 dph		40 dph		8 dph		8 dph	
Exposure Water	Lab.	C.R.	Lab.	C.R.	Lab.	C.R.	Lab.	C.R.	Lab.	C.R.	Lab.	C.R.
Hardness	66	65	54	59	59	57	53	58	76	72	76	72
LC50	22 (20-25)	25 (22-29)	9 (7-12)	18 (15-22)	177 (154-186)	> 410 [i]	528 (439-618)	1556 (1101-2200)	14.5 (13.7-15.3)	72 (61-83)	150 (141-159)	625 (505-773)
LC50 - Hardness Adjusted [a]	22		10		169		592		13.7		143	
Water Effects Ratio (WER)	1.1		1.8		n/a		2.6		5.3		4.4	
CWQG	2	2	2	2	1	1	1	1	0.03	0.03	30	30
CWQG WER Adjusted [b]		2.2		3.6		n/a		2.6		0.16		132
EPA Water Quality Criteria (WQC)	10	8	8	10	36	35	32	36	1.4	1.4	93	89
EPA WQC WER Adjusted [c]		13		11				94		7.4		392
WA WQC	12	11	10	10	36	35	32	36	1.7	1.6	91	87
WA WQC WER Adjusted [d]		12		18				94		8.5		383
LC50 - BLM Normalized to Columbia River Water Chemistry [e]		16 (± 2)		17 (± 4)		244 (± 10)		917 (± 85)		9.7 (± 0.4)		130 (± 10)
LC50 - BLM Normalized to EPA Reference Water Chemistry [f]	4.5	8.1	4.0	4.3	98	> 219 [i]	600	1087	11	33	100	945
BLM Normalized Species Mean Acute Value (SMAV) [g]	4.6				400				19		307	
EPA WQC based on EPA Reference Water Chemistry [h]	2.3				54				1.7		102	

Values in parentheses for LCs represent 95 % CI, values in parentheses for Biotic Ligand Model (BLM) predictions represent standard deviation; WER = water effect ratio; CWQG = Canadian water quality guideline; EPA WQC = environmental protection agency water quality criteria; WA WQC = Washington State water quality criteria.

[a] LC50 Hardness Adjusted refers to LC50 values from laboratory water exposures adjusted to the hardness of the corresponding river water exposures

[b] CWQG WER Adjusted refers to the Canadian Water Quality Guidelines following application of WERs

[c] EPA WQC WER Adjusted refers to US Environmental Protection Agency water quality criteria following application of WERs

[d] WA WQC WER Adjusted refers to Washington State water quality criteria following application of WERs

[e] LC50-BLM Normalized to Columbia River Water Chemistry refers to LC50 values from laboratory water exposures normalized to the water quality parameters of the corresponding river water exposures by use of the BLM and following the procedures outlined in the EPA water quality criteria document for Cu (EPA 2007)

[f] LC50-BLM Normalized to EPA Reference Water Chemistry refers to LC50 values normalized to the water quality parameters of the EPA reference water chemistry outlined in the EPA water quality criteria document for Cu (EPA 2007) by use of the BLM

[g] BLM Normalized Species Mean Acute Value (SMAV) refers to the species mean (geomean) acute value calculated from LC50s that have been normalized to the water quality parameters of the EPA reference water chemistry outlined in the EPA water quality criteria document for Cu (EPA 2007) by use of the BLM

[h] EPA WQC based on EPA Reference Water Chemistry refers to US Environmental Protection Agency water quality criteria calculated for the water quality parameters of the EPA reference water chemistry outlined in the EPA water quality criteria document for Cu (EPA 2007) by use of the BLM for Cu and hardness regression equations for Pb, Cd, and Zn

[i] LC50 value represented as "greater than" due to insufficient mortality

organic carbon (TOC) measurements instead of DOC. TOC measurements were used as an alternative when it was confirmed through separate third party sampling and analyses that the TOC and DOC varied little in the sampled water sources.

3.4.3 Lethal Concentrations

Dose response relationships and median lethal concentrations (Figure 3.1 and Table 3.3, respectively) were calculated for all life stages and metals tested, with the exception of white sturgeon 8 dph exposed to Pb in river water, where the greatest concentration tested resulted in 98% survival. Average survival of unexposed control fish was 90% or greater in all experiments. Results for exposures to Cu in laboratory water have been previously reported (Vardy et al. 2013). LC50s were consistently less in laboratory water than river water, and as a result, WERs were greater than 1 in all cases (Table 3.3). At 8 dph, however, white sturgeon exhibited comparable sensitivity to Cu in laboratory and river water, with LC50s of 22 and 25 µg Cu/L, respectively, and a WER slightly greater than one (1.1, Table 3.3). In contrast, at 40 dph white sturgeon were twice as sensitive to Cu in laboratory water as they were in river water. For 8 dph exposures, white sturgeon were approximately four and five times more sensitive to Zn and Cd, respectively, in laboratory water than river water. Due to a lack of mortality caused by Pb in river water at 8 dph, no sensitivity comparisons could be made. White sturgeon displayed differences in sensitivity between life stages when exposed to the same metal. For Pb, white sturgeon were three and a half times more sensitive when exposed in laboratory water at 8 dph than at 40 dph. In contrast, an opposite trend towards greater sensitivity to Cu at 40 dph

compared to 8 dph was observed in both laboratory and river water exposures. Overall, white sturgeon were more sensitive to Cd and Cu and less sensitive to Zn and Pb.

Based on the results of exposures in laboratory water and Columbia River water, BLM sensitivity parameters (i.e. LA50s) for Cu, Pb, Cd, and Zn were determined for white sturgeon early life stages (Table 3.3). LA50s were consistently greater in river water exposures compared

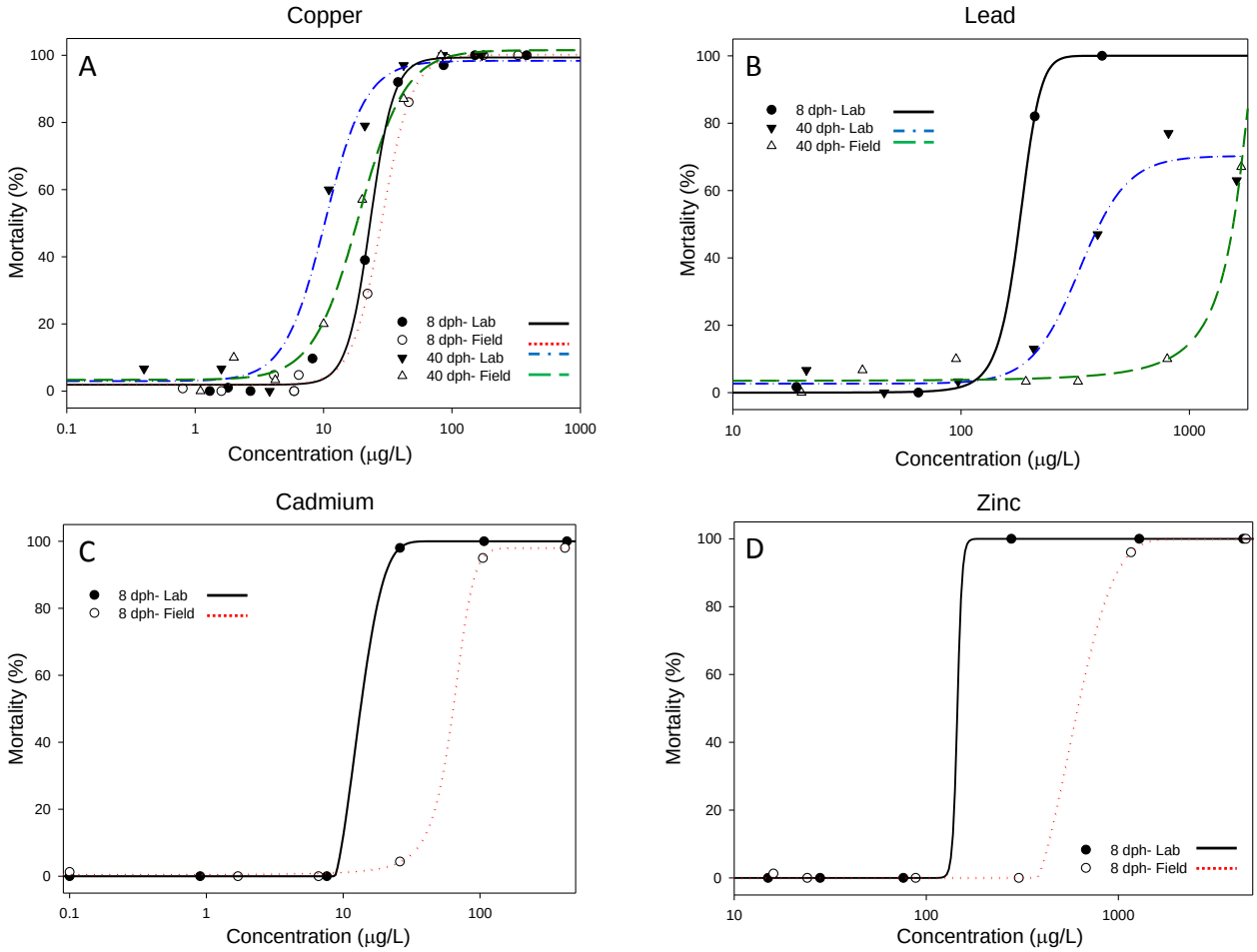

Figure 3.1. Dose response relationships for early life stages (8 days post hatch [dph] and 40 dph) of white sturgeon exposed to copper (A), lead (B), cadmium (C), or zinc (D) in laboratory water (lab) and Columbia River water (field).

to laboratory exposures in all paired tests. When the BLM was used to normalize laboratory based LC50s to field conditions using the water quality data from the corresponding river water exposure, BLM predictions were significantly less than the observed LC50s in Columbia River water in all cases. With the exception, however, for 40 dph white sturgeon exposed to Cu where predictions and observed LC50s overlapped. BLM calculations for 8 dph white sturgeon exposed to Cu were based on results from the additional set of exposures that were conducted (section 3.3.5) due to the increased sampling of water quality parameters.

Water quality criteria for the US EPA and the state of Washington, as well as Canadian WQGs, were calculated for the different experiments (Table 3.3). With the exception of 40 dph white sturgeon exposed to Cu in laboratory water, where criteria and threshold values overlapped, WQC/WQGs calculated for laboratory and river water were less than LC50s for white sturgeon.

3.5 Discussion

Thresholds for effects of several metals of concern to early life stage white sturgeon were determined in acute exposures. Results of previous studies have shown that early life stage white sturgeon are among the most sensitive fishes to effects of Cu, as illustrated by species sensitivity distributions (Vardy et al. 2011, 2012). To assess the relative sensitivity of early life stage white sturgeon to Pb, Cd, or Zn, threshold values were compared to those of trout (*Oncorhynchus*) and sculpins (*Cottus*), species that are often considered sensitive to metal exposure (Besser et al. 2007; Teather and Parrott 2006). For comparative purposes, all thresholds from other studies (Besser et al. 2007; Hansen et al. 2002; Mebane et al. 2012) presented herein were adjusted to

the same water hardness as those from the present study. In the current study, LC50s for white sturgeon exposed to Pb in laboratory water were significantly less than average LC50s for rainbow trout (*Oncorhynchus mykiss*; 1,190 µg/L), and less than or equal to those for cutthroat trout (*Oncorhynchus clarki lewisi;* 493 µg/L), that were calculated from a series of comparable experiments (Mebane et al. 2012). However, for Cd an opposite trend was observed whereby early life stage white sturgeon exposed to Cd in laboratory water were less sensitive than rainbow trout, bull trout (*Salvelinus confluentus*), cutthroat trout, shorthead sculpins (*Cottus confuses),* and mottled sculpins (*Cottus bairdi*), all of which exhibited LC50s that ranged between 1.7 and 3.5 µg/L (Besser et al. 2007; Hansen et al. 2002; Mebane et al. 2012). Thresholds for mortality of white sturgeon exposed to zinc were more consistent with those of rainbow trout, bull trout, and mottled sculpins (range between 114 and 251 µg/L), and approximately three times less than those of cutthroat trout and shorthead sculpins (494 and 528 µg/L, respectively; Besser et al. 2007; Hansen et al. 2002; Mebane et al. 2012).

Sensitivity of early life stage white sturgeon to Cu, Pb, Cd, and Zn appears to be variable under acute exposures in comparison to trout and sculpins. For derivation of WQC/WQGs for freshwater environments, salmonid data are typically required as a minimum because these fishes are considered to be among the most sensitive (CCME 2007; Stephan et al. 1985). Consequently, the protectiveness of current WQC/WQGs for metal toxicity to early life stage white sturgeon is unclear. Based on the water quality parameters from the laboratory exposures from the present study, the Canadian WQGs for the protection of aquatic life for Cu, Pb, Cd, and Zn, are less than the most sensitive LC50s obtained in this study (Table 3.3; CCME 2013). In comparison, LC50s were also greater than United States EPA national acute criteria for aquatic

life (CMC) and acute criteria for the state of Washington for Pb, Cd, Zn, and the 8 dph Cu life stage, but bordered criteria for the 40 dph Cu life stage (Table 3.3; EPA 1987, 2001, 2007, 2009; WAC 2012). Concerns with Cu threshold values approaching US criteria during white sturgeon swim-up life stages have been previously addressed (Vardy et al. 2012).

Further investigation into site-specific conditions that may affect the bioavailability of metals or the sensitivity of fish to metals is merited in water bodies inhabited by sturgeon. To investigate the potential risks associated with metal exposure to early life stage white sturgeon in the Columbia River, WERs and LA50s were developed. WERs were consistently > 1 indicating that metals were less toxic to white sturgeon when exposed in Columbia River water compared to laboratory water. This could, in part, be due to river water containing greater organic matter, suspended particles, or other constituents such as Ca^{2+}, that can bind or compete with divalent metal ions and reduce bioavailability and in turn toxicity. Columbia River water and laboratory water had similar DOC, pH, and total hardness, yet the Ca^{2+} to Mg^{2+} ratio was much greater in Columbia River water (~ 4:1) compared to laboratory water (~ 1.3:1). Previous studies have suggested that the protective effects of hardness from toxicity of metals, such as Cu, are largely dictated by Ca^{2+} and not Mg^{2+} (Naddy et al. 2002; Santore et al. 2001; Welsh et al. 2000b). At the gill, the primary site of action in acute toxicity of heavy metals in fish, the binding of Ca^{2+} is greater than Mg^{2+} (Santore 2001). As a result, greater concentrations of Ca^{2+} can lead to greater competition with metals such as Cu and in turn can reduce toxicity, which may explain the decreased toxicity in Columbia River water.

Results from the BLM, however, suggest that the observed differences in toxicity between river water exposures and laboratory water exposures are not entirely due to differences in water quality and metal bioavailability, but rather in combination with differences in fish sensitivity. The BLM utilizes site specific water quality parameters to account for bioavailability of metals when deriving toxicity predictions. In the present study the BLM was used to normalize LC50s in laboratory water exposures to river water exposure conditions (see methods section 3.3.5). Normalization of values adjusts for differences in water quality characteristics between studies and allows for more accurate comparisons. In the same manner, the BLM can also be used as a predictive toxicity tool in risk assessment when water quality parameters of water bodies of interest are known. In the present study, BLM normalized LC50s were less than the observed LC50s in Columbia River water (Table 3.3), suggesting that other factors besides bioavailability might be affecting toxicity to sturgeon. In addition, the LA50s calculated for sturgeon exposed in river water were always greater than the corresponding LA50 for sturgeon of the same life stage exposed to the same metal in laboratory water. If differences in observed LC50s between paired exposures were due solely to differences in water chemistry then the BLM should account for this and the LA50s would be similar.

It is therefore hypothesized that sturgeon cultured and exposed in laboratory water, with lesser concentrations of Ca^{2+} in comparison to river water conditions, might have been less tolerant to metal toxicity. Previous studies have reported the influence of Ca^{2+} in culture water having a carry-over effect in subsequent exposures and altering the sensitivity of fishes to metals (Barron and Albeke 2000; Erickson et al. 1997; Mebane et al. 2010; Naddy et al. 2003; Welsh et al. 2000a). Although results vary between studies, it has been proposed that fish incubated in

water with lesser concentrations of Ca^{2+} have greater energy demands to prevent the loss of Ca^{2+} through the gill and maintain homeostasis, and in the process become more susceptible to metal toxicity, and are thereby more sensitive during subsequent exposure to metals (Mebane et al. 2010). As demonstrated in the present study, significant differences in Ca^{2+} to Mg^{2+} ratios between laboratory waters and field conditions could have implications when deriving site specific water quality objectives and lead to over or under protective standards.

BLM LC50 predictions for Cd and Zn were not in close proximity to observed LC50s in Columbia River water. In addition to the above mentioned hypotheses, this could, in part, be a function of the dose-spacing (4-fold increments between doses) and the fact that there was little to almost complete mortality between the third and fourth doses in both Cd and Zn exposures, which leads to some degree of uncertainty when calculating LC50s, and in turn, WERs. In addition, concentrations of Cd and Zn in river water were only measured in stock solutions, and therefore, the true concentrations of metals in exposure chambers are unknown. Likewise, these uncertainties could also lead to inaccuracies when modifying BLM parameter files for white sturgeon and lead to ambiguity when calculating LA50s and in turn model predictions. Further data collection, under varying field conditions, is needed before any definitive conclusions can be made.

In a comparable study with white sturgeon, Little et al. (2012) investigated the effects of Cu toxicity. For comparison of LC50s between studies, values calculated from Cu exposures in laboratory water and river water from the present study were normalized to the EPA reference water chemistry outlined in the EPA WQC document for Cu (EPA 2007), following the same

normalization procedures carried out by Little et al. (2012). The geomean of normalized LC50s from the present study compared well with the geomean of normalized LC50s from Little et al. (2012) for white sturgeon of a similar life stage at approximately 40 dph (4.1 μg/L and 3.6 μg/L, respectively). In addition, to better assess the protectiveness of EPA WQC, the species mean acute value (SMAV) was calculated for early life stages of white sturgeon between 8 and 45 dph from LC50s from the present study, a previous study with white sturgeon exposed to Cu (Vardy et al. 2012), and from Little et al. (2012; Table 3.3). All LC50s were normalized to EPA reference water chemistry as previously described by use of the BLM. The resulting SMAV for white sturgeon exposed to Cu (4.6 μg/L) is greater than the EPA WQC for reference water chemistry (2.3 μg/L). A more conservative approach can be used to assess the degree of protection by calculating one half the species mean acute value (½ SMAV). This is similar, but on a species level, to EPA WQC methods where one half final acute values (FAVs) are calculated. ½ SMAV for white sturgeon exposed to Cu (2.3 μg/L) is equal to EPA WQC for reference water chemistry, indicating borderline protection. The same normalization procedures were performed with LC50s for Pb, Cd, and Zn from the present study and SMAVs were developed (Table 3.3). When compared to the hardness adjusted EPA WQC for EPA reference water chemistry, SMAVs and ½ SMAVs were greater than standards (Table 3.3). When compared to Washington State WQC for reference water chemistry, however, SMAVs and ½ SMAVs were significantly less than standards for Cu (15 μg/L), indicating possible issues with inadequate protection. Washington State WQC for reference water chemistry for Pb, Cd, and Zn, and Canadian WQGs for reference water chemistry for all four metals of concern were less than ½ SMAVs for early life stage white sturgeon, indicating acceptable levels of protection.

There are a limited number of methods that are typically used to calculate site specific numerical water quality objectives, including hardness regression equations, water effect ratios, and free ion activity modeling, as illustrated in the present study. In some instances, the sensitivity of specific resident species may be considered, and guidelines and criteria modified to ensure adequate protection (CCME 2007; Stephan et al. 1985). Currently, however, no definitive method for derivation of water quality objectives has proven to stand alone, all have strengths and limitations. In Canada, freshwater guidelines for the protection of aquatic life for metals of environmental concern are typically adjusted for hardness, although many of these guidelines were developed over twenty five years ago and are in need of updating. Guidelines for Zn, for example, are simply set a 30 μg/L without adjustments, even though it is recognized that Zn toxicity is hardness dependent (CCME 2007, 2013). In Europe and the United States, use of predictive modeling to help develop water quality objectives that are more site specific is becoming more common, although challenges remain when trying to account for the interactions of the variables typically encountered in a dynamic, real world, environment. WERs are useful tools that allow for the incorporation of site specific water characteristics as a whole into water quality standards, but the process can be time consuming and costly and often temporal variability is not taken into account (CCME 2007). It should be noted that guidance documents for the development of WERs usually require bioassays from more than one species, typically an invertebrate and a fish, often include a standard test species, and require culturing of organisms in comparable waters prior to test initiation, to be incorporated into calculations of WERs (Stephan et al. 1994). Fish in the present study, however, were incubated and cultured in

laboratory and river water prior to their corresponding exposures to elucidate possible differences in sensitivity that fish might acquire in their natural environment.

The results of the present study demonstrated differences in toxic potency of metals in laboratory and river water. Adjusting thresholds determined in laboratory water to the hardness of river water did little to account for differences in observed mortalities. Incorporating the other water quality parameters of the BLM into adjustments through normalization reduced the differences between threshold values in some cases but also resulted in greater disparities in others. This demonstrated that sole adjustments for hardness inadequately relates laboratory based exposures to field conditions and that BLM normalization may not account for differences in acquired sensitivities of fish during rearing in different environments. In Canada, guidelines are significantly less than US criteria and it may be argued that they are overprotective. In contrast, simple hardness adjustments employed to calculate standards in the state of Washington may result in WQC that are under-protective. Use of the BLM for Cu criteria appears to function relatively well and revision of current Canadian WQGs and US criteria to adopt the current BLM practices employed by the EPA for Cu appears merited. Concern over the degree of protectiveness of metal related water quality standards for early life stage white sturgeon is a valid concern and further research is warranted. Criteria and guidelines should be evaluated closely in areas inhabited by white sturgeon.

CHAPTER 4

Previous work determined that early life stages of white sturgeon were relatively sensitive to metals under acute and chronic exposure conditions (Chapter 2, 3, and Vardy et al. 2011, 2013, 2014a). In the Upper Columbia River (UCR), water quality criteria are providing adequate protection for sturgeon from metals such as cadmium, lead, and zinc, yet only borderline protection from copper. Teck, a metallurgical company, operates a smelter facility in Trail, BC, Canada, and releases effluent into the UCR that is elevated in these primary metals of concern. Previous laboratory-based experiments with early life stage white sturgeon indicate no adverse effects on survival from direct exposure to Teck effluent at environmentally relevant concentrations (Vardy D. MSc Thesis). However, in order to confirm a lack of effects on early life stage white sturgeon in the Columbia River due to discharge of Teck effluent, more information was needed. Previously reported studies did not address uncertainties due to the occurrence of metal mixtures in the UCR or the presence of other factors, chemical or biological, within river water than might influence toxicity to sturgeon under chronic exposure conditions.

To date, little to no information exists with regard to characterizing the potential toxicity of UCR whole water downstream of Teck's Trail facility to white sturgeon. Adult white sturgeon are known to spawn downstream of the metallurgical facility near Waneta Eddy, BC, Canada, and chemicals of interest (COI's) associated with Teck's activities are of potential concern. The purpose of this study was to assess the hatch, survival, and development rates of early life stages of white sturgeon raised in river water upstream and downstream of the Teck Trail smelter facility. In addition, we characterized concentrations of COIs in surface water during the reproductive season of adult white sturgeon, and during the time when early white sturgeon life

stages are present. The following article has been previously published: Tompsett A, Vardy D, Higley E, Doering J, Allan M, Liber K, Hecker M, Giesy JP. 2014. *Effects of Columbia River water on early life stages of white sturgeon (Acipenser transmontanus)*. Ecotoxicology and Environmental Safety. 101: 23-30. A description of co-authorship can be found in Appendix F.

4.0 EFFECTS OF COLUMBIA RIVER WATER ON EARLY LIFE STAGES OF WHITE STURGEON (*ACIPENSER TRANSMONTANUS*)

4.1 Abstract

The white sturgeon (*Acipenser transmontanus*) population that resides in the Columbia River in British Columbia (BC), Canada has suffered recruitment failures for more than three decades. During the summers of 2008 and 2009, studies were performed to determine whether exposure to water downstream of a metal smelter in Trail, BC affected survival or growth of early life stages of white sturgeon through 60+ days post-fertilization (dpf). In both years, there were no significant differences in survival of fish that were exposed to water from downstream compared to upstream of the smelter. At 20-21 dpf, average mortality was 2.4% and 12% in upstream water for 2008 and 2009, respectively, which was similar to average mortality of 3.8% and 7.2% in downstream water for 2008 and 2009, respectively. Relatively great mortality after 20-21 dpf complicated analysis of the subchronic exposure, but use of a survival analysis indicated that the average fish died at 25-29 dpf, regardless of whether the water to which they were exposed came from upstream or downstream of the smelter. In addition, measured concentrations of metals in river water were less than the threshold for adverse effects on white

sturgeon. Based upon these analyses, it is not likely that current concentrations of metals in the Columbia River are adversely affecting survival of white sturgeon larvae.

4.2 **Introduction**

The white sturgeon (*Acipenser transmontanus*) is the largest freshwater fish in North America. However, in recent decades, the number of individuals in most populations of white sturgeon, including the population in the Columbia River between the Hugh L. Keenleyside dam in British Columbia (BC) and the Washington, USA border (Figure 4.1), has decreased. While the specific cause or causes of decreases in the number of adult white sturgeon in the Columbia River are unclear, factors such as historic overharvest and persistent recruitment failures have likely contributed (Irvine et al. 2007; Hildebrand and Parsley 2013). Currently, the population of white sturgeon in the Columbia River is dominated by aging adults, and there is insufficient recruitment of juveniles in the wild to maintain the population into the future (Hildebrand and Parsley 2013). For this reason, this population has been classified as endangered under the *Species at Risk Act (SARA)* and is currently maintained by a hatchery-based stocking program. Reasons for failure of recruitment of juvenile white sturgeon in the Columbia River are not well understood, but previous research has indicated that adult sturgeon in the river spawn and lay viable eggs that hatch at three sites. The major site is near the tailrace of the Waneta Dam in this stretch of the river (Golder Associates Ltd. 2006). However, almost all fry disappear sometime during their first year of life. Little information on the biology, physiology, and appropriate habitat for these early life stages of white sturgeon is available (Coutant 2004), and there is some debate among researchers over the exact timing and sequence of certain behavioral events during

white sturgeon early life stage development, with these events possibly being influenced by differences in availability of appropriate substrata (McAdam 2011).

Figure 4.1. Map of the Columbia River study area.

Map of the Canadian portion of the Columbia River downstream of the Hugh L. Keenleyside Dam. The reach of the river between the Keenleyside dam and the Canada/USA border is approximately 60 km long. Experiments were conducted in two mobile laboratories situated on the east bank of the river. The upstream field site (UFS) was located upstream of the city of Trail and the smelter site. The downstream field site (DFS) was located downstream of the smelter site and upstream of the Canada/USA border.

However, there is a general acceptance that early life stages of white sturgeon are in close contact with the substratum and exhibit distinct hiding and drifting phases (Brannon et al. 1985; Deng et al. 2002; Kynard and Parker 2005). Yolksac stage white sturgeon tend to hide/burrow in refugia (Brannon et al. 1985; Gessner et al. 2009; McAdam 2011; personal observation in the laboratory). Prior to transitioning to exogenous feeding, sturgeon swim up in the water column, presumably to be transported by currents to more suitable foraging grounds (Gessner et al. 2009;

McAdam 2011), before returning to the bottom during the juvenile life stage, where they begin to scavenge and prey on benthic species and spend much of life closely associated with sediments. In general, however, it remains difficult to elucidate factors that are contributing to the failure to recruit juvenile sturgeon. Without identification of the specific factors responsible for the failures, it has not been possible to remediate the cause to allow natural recruitment to the population.

Several factors that have been hypothesized to contribute to lesser recruitment to the Columbia River population of sturgeon include loss of appropriate spawning substratum or areas for fry to develop, alteration of water quality variables and primary productivity due to impoundment of the river by dams, predation by introduced species such as walleye (*Sander vitreus*), failure to recover from past overharvest due to genetic depression, and pollution with nutrients, metals and organic chemicals (Coutant 2004; Kruse and Scarnecchia 2002a; Kruse and Scarnecchia 2002b; Hildebrand and Parsley 2013). The river channel and watershed of the Columbia River have been modified from their historical conditions. There are 14 major hydroelectric dams on the river and 46 in the watershed. The river receives runoff from both urban and rural areas, as well as from various effluent inputs from municipal and industrial sources, including a lead and zinc smelter in Trail, BC, Canada (EPA 2006 a,b). Possible exposure of white sturgeon to contaminants has received some attention in recent years (Bennett and Farrel 1998; Coutant 2004; Feist et al. 2005; Foster et al. 2001 a,b; Kruse and Scarnecchia 2002a; Kruse and Scarnecchia 2002b; MacDonald et al. 1997; UCWSRI 2002). In addition, concentrations of some metals in sediments and other environmental matrices within the Columbia River are elevated (Bortleson et al. 2001; Cox et al. 2005; Majewski et al. 2003;

Paulson et al. 2006). Since it has been suggested by some investigators that these concentrations of metals could have adverse effects on early life stages of white sturgeon, further investigation of the potential effects of exposure of sturgeon to metals in the Columbia River was warranted, especially since the early life stages of some fishes are thought to be most sensitive to the effects of chemicals, including metals (Conte et al. 1988; Hutchinson et al. 1998). Results of a chronic study of the effects of three metals of concern in the Columbia River, copper (Cu), cadmium (Cd), and zinc (Zn), on white sturgeon demonstrated that early life stages of this species are among the fishes that are most sensitive to aqueous exposure to these metals (Vardy et al. 2011). As such, white sturgeon that live in the Columbia River basin upstream of the Grand Coulee dam could be vulnerable to adverse effects.

The objective of the current study was to determine whether metals in Columbia River water could affect survival of early life stages of white sturgeon at their spawning site near the tailrace of the Waneta Dam. This location is the closest early life stages of sturgeon would be expected to be to the smelter and is assumed to be the worst case for potential exposure to metals for this species in the river stretch of concern. Specifically, during the summers of 2008 and 2009, effects of river water on survival and growth of white sturgeon from 8 h post-fertilization through 60-69 days post-fertilization (dpf) were determined. For the duration of the experiments, sturgeon were maintained on-site in water from the Columbia River at locations both upstream and downstream of the smelter in Trail, BC. In addition, concentrations of selected metals of interest were measured in river water during this period to determine whether any metals were present in excess of their respective water quality guidelines and whether concentrations of Cu,

Cd, and Zn, specifically, would be expected to be chronically toxic to early life stages of white sturgeon (Vardy et al. 2011).

4.3 Materials and Methods

4.3.1 Treatment Water Sources

During the summers of 2008 and 2009, white sturgeon were exposed to bottom-near water from two locations along the 60 km long Canadian portion of the Columbia River between the USA border and Hugh L. Keenleyside dam (Figure 4.1) for up to 69 days. Sites were situated both upstream and downstream of a smelter located in Trail, BC, Canada. The upstream field site was situated on the east bank of the Columbia River on the north end of the city of Trail (49°07’01.32”N and 117°43’27.25”W). The downstream field site was located on the east bank of the river north of the Canada-USA border (49°00’28.35”N and 117°36’56.69”W), near the Waneta eddy. Both sites were continuously supplied with water from the river by pumping apparatuses located close to the bottom of the river channel where larvae and fry are expected to live. A control treatment, carbon filtered municipal water from the city of Trail, was also included in both experiments.

4.3.2 Source and Care of White Sturgeon

All research performed complied with the legal standards of the country of Canada and the province of British Columbia. Proper federal and provincial permits were obtained before research commenced (Fisheries and Oceans Canada SARA Permit #SECT 08 SCI 015; British

Columbia Transport Permit #11039), and all work was approved by the University of Saskatchewan's Animal Research Ethics Board (Protocol #20070049).

In both years, adult white sturgeon brood stock were captured at or upstream of the Waneta spawning site in the Columbia River in spring/early summer, and spawned by the Kootenay Trout Hatchery (Forte Steele, BC, Canada). Fertilized eggs were obtained from the hatchery on 15 July 2008 and 14 July 2009. Embryos were introduced to treatment water approximately 8 h post-fertilization. Embryos for each replicate were incubated in a small egg hatching jar (Aquatic Ecosystems, Apopka, FL, USA) attached to a flow-through system. After hatching was complete, yolk-sac larvae from each replicate were enumerated and separated into three experimental chambers. In 2008, 358 ± 122 fish were stocked into each chamber initially, and in 2009, 222 ± 48 fish were stocked into each chamber initially. The relatively great variability in the number of fish stocked per chamber was due to difficulties encountered in accurately separating and counting the small, delicate fry. The lower stocking rates in 2009 were utilized to minimize larval mortality.

When resorption of yolk-sacs was nearing completion, food was introduced to the chambers to acclimate larvae to its presence. In 2008, larval sturgeon were fed freshly hatched brine shrimp (*Artemia spp.*) (1x/d) and a paste made from frozen bloodworms (Hagen, San Francisco Bay Brand) (3x/d) *ad libitum*, for a total of four feedings per day. During the 2009 study, sturgeon were fed freshly hatched brine shrimp 1x/d and bloodworms 4-5x/d *ad libitum* during the day. In addition, sturgeon were fed Bio Diet semi-moist feed (equal parts #0 trout chow, cyclopeze, krill, and tubifex) overnight at 2 hr intervals by use of automatic feeders. In

both years, mortalities were determined daily. In 2008, larvae were grown to 60-61 dpf in treatment water; the experiment was terminated on 13-14 September. In 2009, larvae were grown to 68-69 dpf, and the experiment was terminated on 20-21 September. At termination of the experiment, sturgeon were euthanized by use of 100 mg MS-222/L, each individual was given a unique identifying number, and mass and length of each individual were determined.

4.3.3 Experimental Design

Experiments were conducted at the riverside in retrofitted commercial trailers (Britco, Langley, BC, Canada). Briefly, river water or filtered city water was continuously pumped into flow-through systems in the trailers (Appendix C, Supplemental Materials Figure C1). Each system consisted of two pumps, an 85 L reservoir, and three 40 L experimental chambers. To sustain appropriate flow-rates, water was both renewed and re-circulated within each system. Flow-through conditions were set such that, on average, one complete water replacement in each exposure system occurred every 6 h. Each treatment was conducted in 4 replicated systems. Average water temperature was maintained via the use of a titanium water chiller in each reservoir, and the light:dark cycle was set at 16h:8h. Water was circulated through the systems for approximately one month prior to the experiments to both flush the systems and to initiate, establish, and maintain a steady-state condition prior to initiating the toxicity tests. This time period also provided an opportunity for natural biofilm-forming microorganisms to colonize the experimental chambers which, based on personal observations, represented an important initial food source for sturgeon during the transition to exogenous feeding.

4.3.4 Water Quality Measurements

Routine water quality parameters, including temperature, pH, dissolved oxygen (DO), and conductivity, were recorded daily by use of Symphony electrodes (VWR International). Weekly measures of hardness, alkalinity, ammonia, nitrate, nitrite, chlorine, sulfate, sulfide, and phosphate were made for each replicate system using colorimetric and titrator test kits (LaMotte, Chestertown, MD, USA) throughout the duration of the study. In addition, during the 2009 study, hardness, alkalinity, sulfate, and ammonia were measured by Columbia Analytical Services (CAS, Kelso, Washington, USA) by use of methods SM 2340C, SM 2320B, EPA 300.0 and SM 5310C, respectively. Values of all water quality parameters were within the range acceptable for fish culture (Table 4.1).

Table 4.1. Mean ± standard deviation water quality parameters in control and waters of upstream and downstream locations of Teck in 2008 and 2009.

Treatment		**Control**		**Upstream**		**Downstream**	
Year		2008	2009	2008	2009	2008	2009
Parameter	**Units**	**Mean ± SD**					
Temperature	˚C	15 ± 0.9	15 ± 0.6	15 ± 0.6	15 ± 0.4	15 ± 0.4	15 ± 0.4
Dissolved oxygen	% saturation	96 ± 11	95 ± 17	96 ± 11	96 ± 16	93 ± 7.1	99 ± 14
pH	su	7.6 ± 0.4	7.5 ± 0.6	7.7 ± 0.3	7.7 ± 0.6	7.8 ± 0.2	7.5 ± 0.7
Conductivity	µS/cm	150 ± 6	130 ± 9	140 ± 5	130 ± 9	110 ± 3	130 ± 9
Ammonia	mg/L	0.056 ± 0.1[a]	0.03 ± 0.025	0.067 ± 0.15[a]	0.03 ± 0.03	0.073 ± 0.12[a]	0.01 ± 0[a]
Nitrate	mg/L	0.13 ± 0.021[a]	0.39 ± 0.46	0.17 ± 0.08[a]	0.32 ± 0.34	0.16 ± 0.063[a]	0.46 ± 0.78
Nitrite	mg/L	0.01 ± 0.002[a]	0.01 ± 0.01[a]	0.01 ± 0.00[a]	0.01 ± 0[a]	0.01 ± 0[a]	0.01 ± 0[a]
Hardness	mg/L	72 ± 10	57 ± 2	72 ± 10	58 ± 4	72 ± 10	57 ± 3
Alkalinity	mg/L	53 ± 6	54 ± 6	57 ± 6	57 ± 6	56 ± 5	59 ± 5
Sulfate	mg/L	ND[b]	NM[c]	0.35 ± 2.6[a]	NM	ND	NM
Phosphate	mg/L	0.025 ± 0[a]	NM	0.025 ± 0[a]	NM	0.025 ± 0[a]	NM
Total Chlorine	mg/L	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL

[a] Most or all measurements were below the method detection limit and were assigned a value of one-half of the detection limit.
[b] ND=Not detected; [c] NM=Not measured; [d] <MDL=<Method detection limit of 0.2 mg total chlorine/L

Samples of each type of exposure water were collected weekly, and concentrations of metals and metalloids, including aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), selenium (Se), silver (Ag), thallium (Tl), and zinc (Zn), were determined. In 2008, all samples were filtered, and dissolved concentrations of metals were measured. In 2009, paired samples were utilized so that both dissolved and total concentrations of metals could be evaluated. Samples of water were collected using acid-cleaned polyethylene bottles. Samples for evaluation of dissolved metal concentrations were filtered through a 0.45 µm polycarbonate filter with Nalgene filter holders and receivers. Samples for both total and dissolved concentrations of metals were acidified with ultrapure nitric acid to a pH <2 standard units, and maintained at approximately 4°C for shipment to the analytical laboratories. In 2008, quantifications of metals were conducted at the Toxicology Centre (University of Saskatchewan, Saskatoon, SK) by use of inductively coupled plasma-mass spectrometry (ICP-MS) following US Environmental Protection Agency (USEPA) Method ILM05.2D. In 2009, concentrations of metals were determined by CAS using ICP-MS following EPA Method 6020.

4.3.5 Statistical Analyses

All statistics were performed using Systat 12 software (Chicago, IL) or SPSS 19 software (IBM, Armonk, NY). Statistical significance was defined as $p \leq 0.05$. Before using statistical tests to determine differences among treatment groups, all data were subjected to Shapiro-Wilk tests of normality and Levene's tests of homogeneity of variances to assure appropriate application of parametric and nonparametric statistical techniques.

Mortality was evaluated on the basis of percent of fish surviving at 21/20 dpf. Since there was elevated mortality between 21 and 60 dpf, it was necessary to consider this confounding factor during analyses. Thus, to improve evaluation and usability of data, a survival analysis was used to evaluate mortality in a manner that was not unduly influenced by the mortalities after 21/20 dpf (Newman and Aplin 1992). This technique also controlled for other aspects of the study design, such as unequal stocking densities. Survival analysis allowed estimation of the average number of days each fish survived without the need to reduce mortality to percent survival which was inherently a function of the initial number of fish stocked per chamber. This approach also provided a basis for making statistical comparisons of survival among treatment groups throughout the experiment.

Specifically, the Kaplan-Meier method was used to provide survival estimates during the course of the study. The Kaplan-Meier method operates on the number of fish at risk at any given time point, and if a fish is removed for any reason (other than death), it is not counted as a mortality event, but rather, it decreases the number of fish at risk of dying at subsequent time points. Mathematically, the Kaplan-Meier method is a product limit formula, and can be described by (Equation 4.1):

$$\hat{S}(t_{(j)}) = \prod_{i=1}^{j} \hat{\Pr}[T > t_{(i)} \mid T \geq t_{(i)}] \qquad \text{(Equation 4.1)}$$

Where:

$S(t_{(j)})$ = estimated survival probability at time *j*,

i = index of multiplication,

T = survival time, and

t = time-point of interest.

In practice, $S(t_{(j)})$ can be determined directly from the survival data (Equation 4.2):

$$\hat{S}(t_{(j)}) = \prod_{i=1}^{j} \left(\frac{n_i - d_i}{n_i} \right) \qquad \text{(Equation 4.2)}$$

Where:

d_i = number of deaths at time i, and

n_i = number or organisms at risk of dying at time i.

For survival until 21/20 dpf, overall number of days of survival, number of fish per chamber at termination of exposure, mass and length of fish at exposure termination, and water metal concentrations comparisons among treatment means were performed via one-way ANOVAs or Kruskal-Wallis tests, for data that met the assumptions for application of parametric statistical procedures and those where non-parametric procedures were appropriate, respectively. Parametric data were subjected to post-hoc Tukey's tests and data that did not meet the assumptions of parametric statistical techniques were analyzed with post-hoc Mann-Whitney U tests. Treatment means are expressed throughout as mean ± 1 standard deviation. All calculations were performed using individual chambers as replicates, with the exception of concentrations of metals where calculations were performed using each system as a whole.

4.4 Results

4.4.1 Metal Concentrations in Columbia River Water

Concentrations of most metals were near method detection limits (Table 4.2). In the context of the current experiment, dissolved concentrations of metal ions were deemed to be of greater biological relevance than total concentrations of metals in river water. Therefore, in 2008 all samples of water were filtered to remove metals associated with particulates. Due to concerns regarding intermittent method-related contamination of blank samples during the filtering process in 2008, in 2009 both total and dissolved concentrations of metals were measured in paired water samples throughout the experiment. Concentrations of metals were equally elevated in both blanks and the paired dissolved samples in a random fashion in 2009. Thus, it is suspected that there was intermittent method-related contamination of water samples from 2008 as well. For this reason, no statistical analyses of the 2008 metal data are presented, and the results presented in Table 4.2 for 2008 should be interpreted with the caveat that concentrations presented here are likely slightly greater than the actual concentrations of metals in the river, and would represent a maximum exposure. The method-related contamination was particularly pronounced for Cd and Zn, so values for those metals are not presented for the studies conducted in 2008. In cases where no contamination occurred, total and dissolved metal concentrations generally did not differ.

In 2009, there were statistically significant differences in mean concentrations of Al, Sb, Ba, Cu, Fe, Pb, and Tl among types of water (Table 4.3), but none of these metals was measured in excess of their applicable water quality guidelines (WQGs). Aluminum and Cd were measured

Table 4.2. Range and median concentrations of metals in control and waters of upstream and downstream locations of Teck in 2008 and 2009, and chronic water quality guidelines for waterborne metals exposures.

Treatment	Control		Upstream		Downstream		**Chronic Water Quality Guideline[b]**
Year	2008	2009	2008	2009	2008	2009	
Metal	**Range and (median) concentrations[a] (µg/L)**						**(µg/L)**
Aluminum	18.5-92.2 (53.3)	77.1-132 (95.2)	<2.7-10.9 (3.3)	6.8-90.3 (11.8)	3.6-11.9 (5.0)	8.4-28.8 (11.7)	100
Antimony	0.063-0.11 (0.079)	0.036-0.27 (0.087)	0.067-0.096 (0.08)	0.033-0.09 (0.043)	0.11-0.28 (0.2)	0.087-0.21 (0.14)	6[c]
Arsenic	0.15-0.67 (0.29)	<0.1-0.4 (0.2)	0.20-0.46 (0.29)	<0.1-0.3 (0.2)	0.2-0.7 (0.33)	<0.1 -0.3 (0.2)	5
Barium	16.4-32.2 (24.2)	19.2-31.2 (22.9)	22.6-27.8 (25.6)	17.5-20 (18.8)	17.5-20.9 (20.2)	17.5-19.9 (18.8)	1000[c]
Cadmium[f]	-	0.013-0.075 (0.026)	-	0.008-0.43 (0.016)	-	0.02-0.049 (0.03)	0.02 or 0.18[d]
Chromium	0.017-0.23 (0.032)	0.05-0.67 (0.09)	0.043-0.094 (0.066)	0.08-1.9 (0.14)	0.049-0.08 (0.064)	0.08-0.23 (0.12)	85[e]
Copper	0.31-1.2 (0.54)	0.26-0.57 (0.36)	0.77-1.3 (1.1)	0.66-1.3 (0.78)	0.53-1.3 (0.98)	0.39-0.65 (0.5)	2
Iron	2.0-18.9 (8.6)	<3.0-15.7 (4.3)	3.2-23.3 (5.4)	<3.0-165 (12.7)	2.7-7.6 (3.7)	3.3-45 (14.5)	300
Lead	0.096-1.2 (0.19)	0.064-0.69 (0.16)	0.071-0.27 (0.11)	0.044-0.70 (0.12)	0.055-1.1 (0.084)	0.057-0.38 (0.13)	1.56
Nickel	<0.5-1.6 (0.64)	0.36-0.70 (0.43)	<0.5-<0.5 (<0.5)	0.38-1.6 (0.51)	<0.5-<0.5 (<0.5)	0.41-2.6 (0.48)	62.35
Selenium	<0.32-<0.32 (<0.32)	<0.30-0.50 (<0.30)	<0.32-<0.32 (<0.32)	<0.30-0.40 (<0.30)	<0.32-<0.32 (<0.32)	<0.30-0.70 (<0.30)	1
Silver	<0.007-0.013 (<0.007)	<0.004-0.02 (<0.004)	<0.007-0.021 (<0.007)	<0.004-0.013 (<0.004)	<0.007-0.03 (0.009)	<0.004-0.019 (<0.004)	0.1
Thallium	<0.002-0.008 (0.003)	<0.005-0.025 (<0.005)	<0.002-0.007 (0.005)	<0.005-0.014 (<0.005)	0.01-0.046 (0.024)	0.006-0.07 (0.03)	0.8
Zinc[f]	-	1.1-3.2 (2.1)	-	0.9-5.0 (1.6)	-	1.0-14 (1.7)	30

[a] Concentrations reported are individually measured values, not replicate averages. Dissolved metals concentrations are reported for 2008 water samples. Total metals concentrations are reported for 2009 samples due to method-related contamination of dissolved metals samples. < indicates that values were less than the Limit of Detection (LOD).

[b] Unless otherwise noted, these values are Canadian Council of Ministers of the Environment-Water Quality Guidelines (CCME-WQG) for the Protection of Aquatic Life. Where appropriate, these values have been calculated based upon the average hardness of river water during the experiments.

[c] No CCME-WQG was available, so the Health Canada Guideline for Drinking Water Quality based on protection of human health is given.

[d] US Environmental Protection Agency Criterion Continuous Concentration (USEPA CCC) for chronic exposures of aquatic life to cadmium based on the average hardness of river water during the experiments.

[e] No CCME-WQG was available, so the USEPA CCC is given.

[f] Zinc and cadmium concentrations from 2008 were omitted due to method-related contamination during sample preparation.

Table 4.3. Mean ± standard deviation concentrations of metals with statistically significant differences among treatments in 2009.

Treatment[a]	CTL	UFS	DFS	**Statistically Significant Differences ($p<0.05$)**
Metal	**Mean concentration ± S.D. (µg/L)**			
Aluminum	97.7 ± 2.4	17.9 ± 2.8	12.8 ± 1.2	CTL > UFS and DFS
Antimony	0.10 ± 0.008	0.05 ± 0.002	0.14 ± 0.001	DFS > CTL > UFS
Barium	23.3 ± 0.4	18.6 ± 0.05	18.6 ± 0.2	CTL > UFS and DFS
Copper	0.37 ± 0.03	0.85 ± 0.04	0.50 ± 0.03	UFS > DFS > CTL
Iron	4.5 ± 1.4	23.9 ± 6.8	15.2 ± 2.5	UFS > DFS > CTL
Lead	0.21 ± 0.04	0.18 ± 0.03	0.13 ± 0.01	CTL > DFS
Thallium	0.003 ± 0.001	0.004 ± 0.001	0.03 ± 0.007	DFS > CTL and UFS

[a] CTL = Control, UFS = Upstream field site, DFS = Downstream field site

in excess of applicable WQGs (Table 4.2), although Al was only detected above guideline concentrations in control water. Cd in all three waters exceeded both Canadian and USEPA standards.

4.4.2 Toxicity of Columbia River Surface Water

Although the studies conducted in 2008 and 2009 were similar, there were some slight modifications in study design between years. Briefly, the average number of fish seeded into each chamber in 2009 was decreased to 222 ± 48 fish from 358 ± 122 fish in 2008 due to optimization of culture conditions for this species (see Appendix C, Supplemental Materials). In addition, the feeding regime was altered to encourage larvae to transition to exogenous food as previously described in section 4.3.2. The 2009 study also was 8 days longer than the 2008 study. While these modifications were minor, they limit the ability to directly compare measurement endpoints between the two years of the study, especially measures of growth. However, the conclusions drawn from the results of the two studies could be compared.

On average, more than 60% of total sturgeon mortalities occurred during the stage of transition to exogenous feeding between 20-21 and 40 dpf, and until the beginning of this transition there was no statistically significant relationship between mortality and initial stocking density (r^2=0.0003, p=0.917 in 2008; r^2=0.01, p=0.556 in 2009). During the first 20-21 dpf, mean mortality among treatments was 2.4 ± 1.4% in 2008 and 7.3 ± 4.2% in 2009, which are both less than the standard of <30% suggested for valid toxicity testing with early life stages of fish (EPA, 1996). Therefore, the results of the study up to 20-21 dpf were considered valid and no correction of the data was needed to evaluate differences among treatments. This smaller data set included observations from fertilization through 21 dpf in 2008 and 20 dpf in 2009. Mean mortalities in 2008 were 1.0% in control water, 2.4% in upstream water, and 3.8% in downstream water, while in 2009, mean mortalities were 3.2% in control water, 12% in upstream water, and 7.2% in downstream water. In both years, before the transition to exogenous feeding, mortality was significantly less in control water than in either river water treatment. There were no significant differences in mortalities between upstream and downstream waters in either year (Figure 4.2).

For the duration of the study, inclusive of fertilization through termination of the experiment, survival analysis was used to determine the mean number of days that a sturgeon that died during the experiment had survived. There were no differences in the mean number of days of survival among treatments (p=0.57 and p=0.13 for 2008 and 2009 data, respectively). Average survival time varied little regardless of treatment with a range of 28-29 and 25-28 d to death in 2008 and 2009, respectively (Figure 4.3).

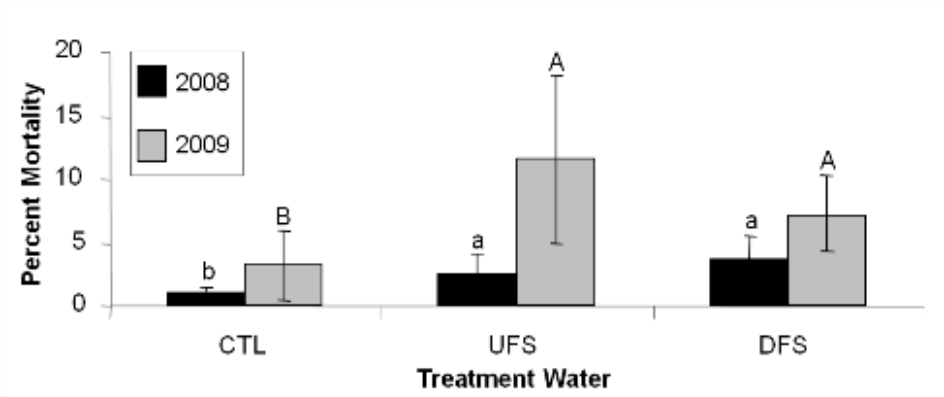

Figure 4.2. Percent mortality of early life stage white sturgeon before transition to exogenous feeding.

Data are presented as mean ± 1 standard deviation (n=4 replicate tanks per treatment). Significant differences are denoted by differing letters. Lower case letters pertain to 2008 data, and upper case letters pertain to 2009 data. No comparisons were made between years.

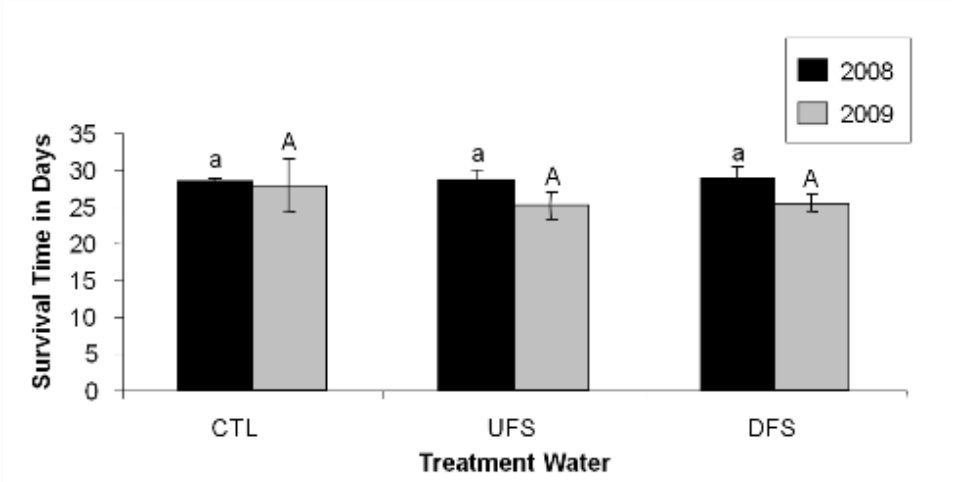

Figure 4.3. Mean survival time in days.

Data are presented as mean ± 1 standard deviation (n=4 replicate tanks per treatment). Significant differences are denoted by differing letters. Lower case letters pertain to 2008 data, and upper case letters pertain to 2009 data. No comparisons were made between years.

Although an unequal number of fish were stocked into the tanks at the initiation of the exposure, the number of fish surviving until the termination of the experiment did not differ among treatments ($p=0.065$ and $p=0.94$ in 2008 and 2009, respectively). In 2008 and 2009, the average number of fish surviving per chamber among treatments was 101 fish and 86 fish, respectively. Within treatments, the number of fish surviving in 2008 was 91 upstream, 105 in controls, and 106 downstream. In 2009, the number of fish surviving was 87 upstream, 85 in controls, and 84 downstream.

4.4.3 Mass and Length of White Sturgeon Early Life Stages

In 2008 and 2009, there were no consistent relationships in mass or length of white sturgeon among treatments. In 2008, there were no statistically significant ($p=0.68$) differences in masses of sturgeon among treatments at the termination of the exposure; mean masses per replicate were 0.38 g in control water, 0.40 g in upstream water, and 0.38 g in downstream water. In 2009, there were statistically significant differences in mass at study termination ($p<0.0001$). Mean masses were 0.74 g in control water, 0.57 g in upstream water, and 0.91 g in downstream water, all of which were significantly different from one another. In 2008, there were no statistically significant ($p=0.91$) differences in mean lengths at termination of the exposure, and mean lengths were 40 mm in all treatments waters. However, in 2009, there were statistically significant ($p<0.0001$) differences in lengths among treatments at termination of the exposure. Fish exposed to either control or downstream waters were significantly longer than those exposed to upstream water. Mean lengths were 51 mm in controls, 46 mm in upstream water, and 54 mm in downstream water.

4.5 Discussion

There is increasing evidence that populations of sturgeons are declining world-wide. This study investigated the potential association between waterborne concentrations of metals in a major North American river system, the Columbia River, and survival and growth of early life stages of white sturgeon. For logistical reasons, it was necessary to culture the sturgeon in mobile laboratories while in the field. This setup allowed for a controlled experiment that utilized river water from both a reference location and a location immediately upstream of the major sturgeon spawning site in the impounded stretch of concern in the Columbia River. The hypothesis that water quality may explain the failure of white sturgeon to survive their first months of life was tested. Based on the results obtained during this study, this hypothesis was rejected. However, it has to be noted that fish were exposed to near-bottom surface water only, and that due to the benthic life-style of yolksac larvae that form mats on the bottom or try to hide in gravel beds, there is also the possibility of exposure to pore-water, which was not tested in this study.

4.5.1 Effects of Exposure of White Sturgeon Early Life Stages to Columbia River Water

Until the period of transition to exogenous feeding (21 dpf in 2008 and 20 dpf in 2009), there were no differences in survival of sturgeon between the upstream and downstream locations. With regard to metal toxicity, the transition to exogenous feeding has been demonstrated to be predictive of the chronic toxicity of Cu, Cd and Zn in white sturgeon (Vardy et al. 2011), and thus, is a relevant time-point for potential metal toxicity assessment in Columbia River water. The greatest mortality of sturgeon occurred between 25 and 40 dpf, regardless of

the water in which they were reared. This period corresponds to the period of transition to exogenous feeding (Coutant, 2004; Boucher 2012) and coincides with the development of a competent immune system in the juvenile fish (Bennet and Farrell, 1998).

Information collected from hatcheries that culture sturgeon indicates that mortality of larvae at the transition to exogenous feeding is a common occurrence and reported mortalities during this life-stage ranged between 50 and 74% (R. Ek, Kootenay Trout Hatchery, Fort Steele, BC, Canada, personal communication; B. Lyon, Columbia Basin Hatchery, Moses Lake, WA, USA, personal communication; J. Van Eenennaam, University of California, Davis, CA, USA, personal communication). When compared to a study by Boucher (2012) mortalities observed at 45dph (end of experimental period in the Boucher [2012] study) in this study were comparable under similar conditions (temperatures between 15 and 18 °C; no substrate provided). However, Boucher (2012) reported improved survival of up to 86% when sturgeon were cultured at lower temperatures and in test systems supplemented with gravel as substrate. The temperature regime used in this study was reflecting temperatures in the Columbia River during the exposure period, and thus, represent a more realistic exposure regime. However, in future studies mortalities could probably be reduced if habitat enrichment in form of substrate is provided. While greater mortality is not considered problematic when rearing sturgeon for commercial or restocking purposes because hatchery managers begin with a sufficient number of fertilized eggs to produce the desired number of fry, mortalities in excess of 30-40% invalidates a toxicity test with early life stages of fish according to current USEPA standards (EPA 1996). However, since white sturgeon are among the most sensitive fish species to the exposure with certain contaminants such as metals (Vardy et al. 2011, 2013) and are classified as threatened or endangered in some

locations, they would be an appropriate species to include in future assessments of environmental conditions, and experimental standards developed specifically for this species would be useful if they are to be used in further toxicity testing.

After transition to exogenous feeding began, there was a statistically significant correlation between initial rates of stocking and mortality of sturgeon (Appendix C, Supplemental Materials Table C2). Therefore, the data for chronic exposures of 60 d 2008 and 69 d in 2009 were evaluated by use of survival analysis, which is a statistical procedure that determines mean days of survival independent of initial stocking rate. Treatment water had no significant effect on the average number of days that a typical fish survived in either 2008 or 2009. Similarly, there were no differences in the number of fish surviving to termination of exposure in either 2008 or 2009. There was some inter-year variability with generally fewer fish surviving to termination in 2009. This result was postulated to be related to changes in study design that were made between 2008 and 2009, which included a reduction in number of fish stocked into each chamber. This reduction improved percent survival, but lessened the number of fish surviving to experiment termination.

Previous studies (Feist et al. 2005; Kruse and Scarnecchia 2002a) have hypothesized that the presence of contaminants in water could be a factor that contributed to failures in recruitment of sturgeon in this reach of the Columbia River. However, the results of the current study indicate that contaminants in the water column downstream of the metal smelter at Trail, BC probably did not affect survival of white sturgeon. In addition, a previous study had reported that the one of the effluents from the metal smelter at Trail, BC, was toxic to juvenile white sturgeon

(exposed from 11 – 14 to 61 - 64 days post hatch) at concentrations of 100% and 50%, but not 1%, resulting in the hypothesis that current liquid inputs from this facility may contribute to the recruitment failures of sturgeon in this reach of the river (Bruno 2004). However, dilution of effluent in the river is such that, at the major spawning site where early life stages of sturgeon are likely to be present where the current study was conducted, there would be no toxicity expected based on the data provided by Bruno (2004).

4.5.2 Concentrations of Metals in the Columbia River

Measured concentrations of metals of concern in waters over the course of the experiment were in accordance with those previously reported for surface water between 2003 and 2005 in the Columbia River at Waneta (CRIEMP 2008), and rarely exceeded CCME-WQGs for the protection of aquatic life (CCME 2013), which are derived to be protective of all species and include a margin of safety. Only two metals, Al and Cd, were measured at concentrations exceeding their respective guidelines (Table 4.2). For all other metals monitored over the course of the study, measured concentrations never exceeded CCME-WQGs.

Concentrations of Al in excess of its guideline (100 µg Al/L) were only measured in control water during 2009, although concentrations in control water in 2008 were as great as 92.2 µg Al/L. The elevated concentration of Al in control water in both years was likely due to the treatment of municipal drinking water for the city of Trail and is not indicative of Al concentrations in the Columbia River. Concentrations reported by Environment Canada for the site at Waneta were similar with values typically not exceeding 40 µg/L and occasional maximum values of up to approximately 145 µg/L (CRIEMP 2008). Since there were no adverse

effects on survival or growth of sturgeon in the control water compared to the river water treatments, it is unlikely that Al at these concentrations is of toxicological concern to sturgeon, even if they were exposed.

There is little evidence that Cd is of chronic toxicological concern to white sturgeon in the Columbia River. Although Cd was detected in excess of the CCME-WQG (0.02 µg Cd/L) and USEPA CCC (0.18 µg Cd/L), the median concentrations of cadmium detected in all water sources (Table 4.2), which would be a more reliable indicator of potential for chronic toxicity, were at least 6-fold less than the USEPA CCC. In addition, concentrations of Cd never exceeded the USEPA criterion maximum concentration (USEPA CMC) for acute exposures of 1.2 µg Cd/L. Concentrations of Cd measured in this study were similar to those previously reported in Columbia River at Waneta (≤ 0.6 µg/L; CRIEMP 2008). All together, measured concentrations of Cd indicate that fish are probably not adversely impacted by exposure to Cd.

Previously derived information on the chronic toxicity of metals to white sturgeon supports the conclusion that chronic toxicity of metals is unlikely to be a concern for sturgeon in the Columbia River. For Cu and Zn, 29 days post-hatch (dph), during the transition to exogenous feeding, was the most sensitive stage during development that was tested, and the chronic lethal concentrations (LC_{20}) for Cu and Zn were reported at 3.4 and 102 µg/L, respectively (Vardy et al. 2011). For Cd, the most sensitive period tested was 59 dph when the LC_{20} was 1.5 µg/L (Vardy et al. 2011). The LC_{20} for Cd, which was the only metal of these three detected in excess of its chronic water quality guidelines in Columbia River water, was 8-fold greater than the USEPA CCC and 75-fold greater than the CCME-WQG. Therefore, both guidelines can be

considered protective of white sturgeon. In addition, the CCME-WQG values for both Cu (2.0 µg/L) and Zn (30 µg/L) would also be protective of white sturgeon. Comparison of concentrations of metals in the Columbia River with concentrations that have been shown to be chronically toxic to early life-stage of white sturgeon (Vardy et al. 2011) indicates that current concentrations of waterborne metals downstream of the metal smelter at Trail, BC are unlikely to be a causative factor for the recruitment failures of sturgeon in the river. However, other sources of pollution cannot be eliminated as potential causative factors. In particular, sediments and organic chemicals could impact survival of sturgeons. These factors were not addressed by this study but are currently being evaluated by our research group. Reasons for decreases in the number of sturgeon in the Columbia River are complex, and it is likely that a combination of factors has resulted in the observed declines. Future studies that simultaneously address multiple factors, such as altered flow-regimes, habitat, water temperatures, and turbidity are needed. Such studies would be difficult and intensive to perform, but would ultimately be needed to understand why populations of these ancient fish have declined over so much of their range.

CHAPTER 5

In comparison to other fishes, early life stage white sturgeon are relatively sensitive to copper, cadmium, zinc, and lead (Chapter 2, 3; Vardy et al. 2011, 2013, 2014a). In the Upper Columbia River (UCR) there are concerns about the effects of metals associated with smelting operations on the resident population of white sturgeon. Investigations into the effects of liquid effluent and whole water toxicity downstream of the smelter on early life stage white sturgeon indicated no adverse effects on survival and growth. Given the epibenthic nature of white sturgeon, however, there is the potential for increased exposure to contaminants through sediments within the UCR. Specifically, there are concerns about the potential toxicity of metals associated with granulated slag, a smelting byproduct that was released into the river in large volumes until 1995, to white sturgeon early life stages, including the early hiding stage where fry are in proximity to sediments. Exposure pathways may include pore water, overlying water, or water at the sediment-water interface. Water may move through the sediments either from upwelling from deeper sediments, downward movement from surface water, or lateral fluvial flow. As part of a Remedial Investigation and Feasibility Study in conjunction with Teck and the U.S. Environmental Protection Agency (www.ucr-rifs.com), sediment toxicity to white sturgeon early life stages was evaluated using field-collected sediments from areas previously hypothesized as suitable white sturgeon habitat, and containing a range of slag-related contaminants of interest (COI) concentrations. Results from this study are presented in two parts. The analytical findings and assessment of metal bioavailability in UCR sediments are the subject of Chapter 5 and will be submitted for publication by Vardy et al. (2014b), whereas Chapter 6 (submitted as Vardy et al. [2014c]) characterizes biological risk from exposure to metals

associated with UCR sediments, and presents the physiological and biological responses of early life stage white sturgeon following UCR sediment exposure. The following article is intended for publication: Vardy D, Doering J, Ryan A, Santore R, Hecker M, Giesy J. Manuscript in preparation. *Assessment of Columbia River sediment toxicity to white sturgeon: concentrations of metals in sediment, porewater, and overlying water.* A description of co-authorship can be found in Appendix F.

5.0 ASSESSMENT OF COLUMBIA RIVER SEDIMENT TOXICITY TO WHITE STURGEON: CONCENTRATIONS OF METALS IN SEDIMENT, PORE WATER, AND OVERLYING WATER

5.1 Abstract

Sediments are sinks for pollutants and can contain contaminants, which can be released back into pore water and the water column following remobilization. Elevated concentrations of trace-elements, such as copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn), relative to reference sites, have been found in sediments downstream of a metallurgical facility in the Upper Columbia River (UCR) in North America. One species of concern with regard to exposure to sediment-born metals in the UCR is the white sturgeon (*Acipenser transmontanus*). Early life stages of sturgeon inhabit benthic habitats, and they live on surface sediments, or in interstitial space between stones. Therefore, they might be at increased risk of exposure to contaminated sediments. In order to assess the risk of exposure to sediment-bound metals in demersal fishes such as sturgeon, bioavailability and concentrations of metals in pore water, overlying water, and at the sediment-water interface need to be characterized. The present study employed a laboratory based, flow-through, experimental exposure system to characterize toxicity of metals

in the different matrices associated with sediments collected in the UCR, and to assess risk to early life stages of white sturgeon under chronic exposure conditions. Several methods including passive sampling (peepers and diffusive gradients in thin films [DGTs]) and active sampling/suction techniques were employed to investigate potential differences in concentrations of metals and other chemical parameters in pore water, overlying water, and at the sediment-water interface. Results indicate that total concentrations of metals in site sediments were significantly greater in comparison to upstream reference sediments. Of the four primary metals of concern, Cu, Cd, Pb, and Zn, concentrations of Cu, primarily in pore water, were significantly greater in exposure chambers containing site sediments compared to reference sediments. Active and passive sampling techniques resulted in similar concentration estimates of Cu for a given sediment, and relatively similar measurements for concentrations of Zn, in pore water and at the sediment-water interface. In contrast, the different sampling techniques often resulted in different estimates of concentrations of other metals analyzed within the matrices, such as Cd and Pb, with a tendency towards greater concentrations in peepers compared to DGTs. The present study highlights challenges in characterizing bioavailability of metals associated with sediment because measured concentrations can vary depending on the choice of sampling method, matrix, and analyte. The analytical data reported herein is utilized in Chapter 6 and a parallel article to characterize risk, and in turn, compare predictions to the biological results from exposure of early life stage white sturgeon to UCR sediments.

5.2 Introduction

Alteration of habitat, including pollution, is hypothesized as a major contributing factor to the global decline of populations of sturgeons (Birstein 1993; Gisbert and Williot 2002; Hu et al. 2009; Irvine et al. 2007; Luk'yanenko et al. 1999; Paragamian and Hansen 2008). Given their epi-benthic nature, sturgeon are potentially at risk of exposure to contaminants associated with sediments. In the Upper Columbia River (UCR), between Grand Coulee Dam in the USA and Hugh L. Keenleyside Dam in southern British Columbia, Canada, resides a population of fewer than 2500 white sturgeon (*Acipenser transmontanus*) that have been experiencing poor recruitment for over forty years (Hildebrand et al. 1999, 2013; Irvine et al. 2007). Although specific reasons for their decline are not fully understood, pollution has been hypothesized as a potential contributing factor to the observed recruitment failure (Hildebrand et al. 2013). Specifically, there are concerns that contaminated sediments in the UCR may be bioavailable to sturgeon and that early life stages, including the early hiding stage where fry are in proximity to sediments, may be at risk.

The UCR is subject to multiple sources of pollution, including discharges from pulp and paper mills, wastewater treatment plants, and diverse mining and smelting operations (Hildebrand et al. 2013). In particular, a metallurgical facility in Trail, BC, Canada, historically released slag into the river, and historically and presently releases liquid effluents. Slag is a partially vitreous by-product of the metal refining process and there are concerns about the leaching of metals into water. Elevated concentrations of trace-elements, such as copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn), relative to reference sites, have been found in sediments

downstream of the metallurgical facility (Besser et al. 2008; Bortleson et al. 2001; EPA 2006 a,b). Sediments are sinks for pollutants and can contain elevated concentrations of metals, which can be released back into the water column following remobilization (Salomons et al. 1987; Sullivan and Taylor 2003). Therefore, in addition to exposure to pollutants in the water column, sturgeon might be exposed to contaminants associated with sediments or contaminants released into the sediment-water interface.

In order to assess the risk of the exposure to sediment-bound metals to demersal fishes, such as sturgeon, bioavailability and concentrations of metals in sediment-associated matrices, such as pore water, overlying water, and the sediment-water interface, need to be characterized. Total concentrations of metals in sediments are poor indicators of potential toxicity and risk as a significant proportion of metal might be sequestered and biologically unavailable (Ankley et al. 1994). The bioavailable fraction of a metal is largely dependent on modifying factors of the environment, both abiotic and biotic, that influence the amount of a metal that can interact with biological processes in an organism, and thus, result in toxicity (Fairbrother et al. 2007). Redox potential and pH can greatly affect the chemistry of a sediment-bound metal, governing its distribution between the solid and dissolved phases, and in turn its movement between the various matrices, with dissolved metals in pore water considered to be the most bioavailable (Ankley et al. 1996; Fairbrother et al. 2007). To assess the bioavailability and toxicity of metals associated with sediments various techniques have been employed, several of which include chemical analysis of whole digested sediment samples, or active or passive measurements of concentrations of metals in pore water using centrifugation or sampling devices such as peepers or diffusive gradients in thin films (DGTs).

Previous studies have investigated releases of elements from contaminated sediments in the Columbia River by use of several methods, such as measurements in pore water (interstitial water), overlying water, and supernatants of aggressively tumbled slurries (Paulson et al. 2006; Paulson and Cox 2007). From these studies Paulson and Cox (2007) concluded that under certain conditions releases of elements from sediment could result in concentrations of metals in various matrices that might be toxic to aquatic organisms. However, the Paulson and Cox (2007) study employed techniques to simulate the potential release of metals from sediments under laboratory conditions that are unlikely to be directly applicable to conditions found in the above-mentioned region of concern of the UCR. Therefore, the purpose of the present study was to assess toxicity of contaminants of potential concern (COPC) associated with sediments to early life stage white sturgeon in the UCR by use of a controlled, laboratory, fluvial, exposure that simulated conditions found within the UCR in the vicinity downstream of Trail, BC, Canada. The present study was conducted as part of a remedial investigation and feasibility study (RI/FS) under the oversight of the US EPA (www.ucr-rifs.com), and data obtained from this work will be used to supplement information in a baseline ecological risk assessment (BERA) and overall RI/FS.

In order to accurately assess toxicity of UCR sediments to white sturgeon, a laboratory-based experimental design was needed that captured potential exposure routes of early life stages of white sturgeon to COPC, especially metals in the river, while assessing bioavailability by allowing quantification of chemical parameters in several matrices. An experimental exposure system for exposing aquatic organisms under fluvial conditions was used to expose early life stage white sturgeon in the laboratory to UCR sediments collected from areas considered to be

suitable habitat for white sturgeon, and containing a range of metal concentrations (EPA 2006 a,b).

To assess bioavailability of metals in UCR sediments and overlying waters, the present study employed a variety of sampling techniques. Peepers (Doig and Liber 2009), DGTs (Davison and Zhang 1994), and active sampling/suction techniques were employed as a multiple lines of evidence approach to investigate concentrations of metals and chemical parameters in various the matrices and to compare results of the various methods. Typically, analysis of pore water is achieved through either active or passive sampling, such as centrifuged and filtered core samples or use of membranes and dialysis chambers (peepers). Because disturbances of sediments during sampling of pore waters have been found to alter chemistries of sediments and affect bioavailability (Murdoch et al. 1997), in the present study peepers were employed to minimize disruption of sediment during sampling and extraction. In addition, peepers enable direct comparisons of concentrations of metals in pore water and overlying water (Robertson and Liber 2009), which is where early life stages of white sturgeon likely to occur. As a secondary measure to compare concentrations of metals in pore water and at the sediment-water interface, DGT's were employed. DGTs utilize an ion-exchange resin and an ion-permeable gel membrane to quantitatively measure concentrations of metals *in situ* (Davison and Zhang 1994), and are a relatively non-intrusive method of sampling. Finally, active sampling methods through direct collection of overlying water, sediment-water interface water, and pore water, by use of suction techniques with syringes, pipettes, and sediment-embedded airstones, were employed. These sampling techniques allowed for a greater volume of water to be collected from the relevant matrices, which facilitated a larger suite of chemical analyses.

To identify specific data needs in addressing the aforementioned concerns and to establish decision rules for the collection of data, the EPA data quality objective (DQO) process (EPA 2006c) was used for this study. Specific DQOs addressed included survival and growth of white sturgeon reared on sediments from the UCR relative to reference sediments. The present article reports concentrations of metals to which early life stages of white sturgeon could be exposed and bioavailability of metals associated with sediments from the UCR. The responses of white sturgeon are presented separately (Chapter 6; Vardy et al. 2014c).

5.3 Methods

5.3.1 Site Selection and Collection of Sediments

Locations in the UCR from which sediments were collected, were in areas known to encompass confirmed white sturgeon spawning- and/or nursing-grounds (Howell and McLellan 2006; Weakland et al. 2011), as well as to represent a range of exposure conditions (EPA 2006 a,b). Sampling focused on the reach of the UCR between Kettle Falls (river mile [RM] 703) to the U.S.–Canada border (RM 745), and was intended to represent a gradient of concentrations of COPC in sediments associated with granulated slag. Specifically, the primary COPCs included Cd, Cu, Pb, and Zn (EPA, 2006 a, b). Areas from which samples of sediments were collected included Deadman's Eddy (DME; RM 737), Northport (NP; RM 735), Little Dalles (LD; RM 729), China Bend (CB; RM 723), Upper Marcus Flats (UMF; RM 706), and Lower Marcus Flats (LMF; RM 704; see Appendix D, Supplemental Materials Map D1). Each sampling area contained three distinct sampling locations. Research and ground disturbance permits where obtained prior to sediment sampling and sampling activities were conducted under supervision of

a Cultural Resources Working Group, with archaeological monitoring of ground-disturbing activities by a qualified archaeologist meeting the U.S. Secretary of Interior's Professional Qualification Standards. Reference sediments were collected in three areas located upstream of Trail, British Columbia, Canada. These included Birchbank Eddy (BBE; RM 764), Genelle (GE; RM 766), and Lower Arrow Lakes (LALL; RM 788; see Appendix D, Supplemental Materials Map D2). In addition to site-specific sediments and reference sediments, artificial substratum sediment (Rolf C. Hagen, Inc., Baie d'Urfe, QC, Canada; Aquarium Substratum Item No. 12648) was also used as a negative control (CTRL), as evaluated and selected through method development work (see section 5.3.3; Appendix D, Supplemental Material Order 10).

Surface sediments, defined as the upper 10 to 15 cm (4 to 6 in.) of the sediment column, were collected using a custom-built stainless steel power VanVeen grab sampler that was specifically designed to operate in hard bottom substrata. Depending on sampling success, as much as ten 20-L (5-gal) polyethylene buckets per sampling location (30 buckets per sampling area) were collected to attain the target sediment volume of approximately 200-L (~50 gal) per location. Immediately after collection, sediments were transferred into 20-L decontaminated polyethylene buckets, sealed, and transported in a refrigerated truck (4 °C) to the University of Saskatchewan (UofS), Saskatoon, Saskatchewan, Canada where they were held at 4 °C until initiation of experiments.

Prior to use, composites of each type of sediment were made by thoroughly homogenizing individual samples within an area for "site" sediments collected from the potentially affected areas of the UCR, reference sediments, and control sediments. This was

achieved by use of a Teflon®-lined, cement mixer retrofitted with a high density polyethylene drum and stainless steel paddles, as deemed an appropriate and effective method of mixing through method development work (Appendix D, Supplemental Material Order 1).

5.3.2 Study Design

Prior to initiation of the definitive study, extensive method development work was conducted in order to evaluate and inform critical design components and considerations of specifically designed flow-through, fluvial simulation system for use in sediment toxicity tests with early life stage white sturgeon at the UofS ATRF. Specifically, an experimental exposure system was needed to allow for adjustment of flow velocity, water replacement time, and recirculation frequency, and provide versatility in sampling techniques while maintaining a practical and reproducible fluvial exposure. A full description of method development work, results, and final test design is provided in Appendix D, Supplemental Material.

Exposure chambers were continuous flow-through systems designed and operated at a rate of flow of approximately 20 L/min, with an illumination cycle of 16-light:8-dark (16:8) hrs., and target water temperature of 16 ± 1 °C. Water was both renewed and re-circulated within each system. Flow-through conditions were set such that, on average, one complete water replacement in each exposure system occurred every 6 h. Test water used during the study had a target water hardness of 65 to 70 mg/L as CaCO3 to simulate conditions found in the UCR, and consisted of a 1:1 mixture of de-chlorinated City of Saskatoon water and ATRF reverse osmosis water. The overall study design elements were in accordance with standard American Society for Testing

Materials (ASTM) guidelines for testing early life stages of fish (ASTM 2005), with minor modifications for white sturgeon.

Homogenized sediments were evenly layered at the bottom of dedicated continuous flow-through exposure chambers at a thickness of approximately 2 inches. Replicate exposure chambers were established based on available sediment volume, with up to a maximum of six replicates per sample location. In addition to exposure chambers containing site sediments, reference sediments, or control sediment, a second negative control group (water-only [H_2O] control) was also established and monitored throughout the duration of the study. Six replicates were established for sediments collected from UMF-01, LD-01, and LALL, four replicates from LMF-02 and GE, and two replicates from NP-03. In addition, three replicates from substrata collected above the water line from the gravel bar at Deadman's Eddy (hereafter referred to as "DE") were also included, as there was difficulty in collecting sufficient volumes of site sediments (see results section).

In order to create a pseudo-hyporheic zone, large pebbles (Rolf C. Hagen, Inc. Aquarium Substratum Item No. 12422) were systematically placed in each exposure chamber at approximately 4 stones per 100 cm^2 to fulfill early life stage white sturgeon habitat requirements (Appendix D, Supplemental Material Order 4).

5.3.3 Collection of Water and Pore Water

Concentrations of metals were quantified in overlying water, sediment-water interface water, and pore water to characterize exposure through the various possible aqueous exposure

routes. Of the 42 exposure chambers, 11 were designated as “chemistry-only” (Table 5.1), in which passive sampling devices, such as peepers and DGT probes, were installed and used to obtain additional water quality information within the top ± 1 cm of the sediment-water interface. Given that both DGT probes and peepers require a distinct period of equilibration (2 and 7 days, respectively), and necessitate disturbing the sediment during deployment and retrieval, dedicated “chemistry-only” exposure chambers were used for these measurements. These exposure chambers were seeded with the same number of white sturgeon and treated in the same manner as the regular exposure chambers except for the incorporation of the additional analytical devices. Exposure chambers designated as “chemistry-only” were to ensure that potential stress, if any, resulting from perturbations in deploying and retrieving DGT probes and peepers were not erroneously considered when interpreting effects on white sturgeon.

5.3.4 Direct Sampling of Pore Water and Overlying Water

During placement of sediments/substrata into test chambers, up to eight RENA Micro Bubbler 6-in. ceramic air-stones (Mars Inc. Hackettstown, NJ, USA) were distributed along the length of each exposure chamber for non-intrusive collection of pore water at a depth of approximately 2.5 cm (1 in.) below the sediment surface (Appendix D, Supplemental Material Order 5). Each air-stone was connected to a 15-ml syringe through a port in the side of the exposure chamber that would allow for extraction of pore water (Appendix D, Supplemental Material Order 5). Samples of water were also collected at the sediment-water interface and overlying water via suction by use of high density polyethylene (HDPE) pipettes and syringes, respectively. For the purpose of the present study, sediment-water interface water is defined as

that water located within the boundary between sediment and the overlying water column, within 1 cm above the sediment surface, within respective exposure chambers. Samples of overlying water within exposure chambers were collected within the top 15 cm (6 in.) of the water column.

Table 5.1. Number of replicate exposure chambers per treatment group evaluated during the course of the Columbia River sediment toxicity study.

Treatment Group	No. of Biology Replicates	No. of Chemistry Only Replicates
LMF - 02	3	1
UMF - 01	4	2
NP - 03	2	0
LD - 01	4	2
DME [a]	2	1
GE	3	1
LALL	4	2
Laboratory Control Substrate [b]	4	2
Water Only (No Sediment)	4	0

Notes:
LMF – Lower Marcus Flats
UMF – Upper Marcus Flats
NP – Northport
LD – Little Dalles
DME – Deadman's Eddy
GE – Genelle (reference sediment)
LALL – Lower Arrow Lake (reference sediment)
Chemistry only chambers represent replicates in which peepers and diffusive gradient thin-film [DGT] probes were installed and used to obtain additional water quality information.
[a] substratum as collected above the water line from the gravel bar at DME
[b] artificial substratum sediment (Rolf C. Hagen, Inc., Baie d'Urfe, QC, Canada; Aquarium Substratum Item No. 12648)

5.3.5 Passive Sampling of Pore Water and Sediment-Water Interface

Additional samples of pore water and sediment-water interface were collected by use of alternative passive sampling devices, specifically peepers (Doig and Liber 2007) and DGT

probes (Davison and Zhang 1994). Peepers were obtained from the Liber laboratory, UofS, Saskatoon, SK, Canada, and DGT probes were obtained from DGT Research Ltd, Lancaster, UK. These samples were collected to provide comparative data at the sediment-water interface, and to collect pore water data within the top 1 cm (0.4 in.) of the sediment profile. Samples collected with peepers and DGT probes were collected at the beginning (Day 8), middle (Day 27), and end (Day 57) of the study, following a 2-day (DGT) and a 7-day (peeper) equilibration period. Preparation, placement and retrieval of peepers followed methods described by Doig and Liber (2007). In short, peepers were placed in the sediment so that the top chamber sampled the sediment-water interface and the bottom chamber sampled pore water at approximately a depth of 1 cm. Each chamber was filled with nanopure water and a 0.45-µm polyethersulfone filter membrane (Whatman, Sigma-Aldrich, Oakville, ON, Canada) was used to separate the chambers from the media. Following the 7-day equilibration period, peepers were removed from designated "chemistry-only" exposure chambers and rinsed with deionized water to remove any residual particles from the 0.45-µm membrane surface. Using a plastic tip equipped pipette, peeper membranes were pierced and their contents immediately transferred into pre-cleaned HDPE or polypropylene vials (as provided by Columbia Analytical Services [CAS], Kelso, WA, USA), preserved, and transported to CAS for chemical analysis. Given the limited sample volumes associated with peepers, chemical analyses focused on dissolved metals.

Preparation, placement, and retrieval of DGTs followed methods described by Zhang (2003), with the exception that probes were placed horizontally within the sediments as opposed to vertically, as deemed an acceptable method of sampling water at the sediment-water interface and pore water 1 cm below the sediment surface (personal communication with Zhang H.).

Following the 2-day period of equilibration, DGT probes were carefully extracted from designated “chemistry-only” exposure chambers and rinsed with deionized water, removing any residual particles from the gel surface. Upon removal and rinsing, DGT probes were sliced along the sediment-surface water line using a Teflon® coated blade. Respective top and bottom gel portions were further sliced into three equal stripes, transferred into dedicated 15-mL high-density polypropylene centrifuge tubes, preserved with 5-mL of 1-M HNO3, and transported to CAS for chemical analysis.

5.3.6 Sampling of Sediments

Following homogenization and prior to placement in the exposure chambers at the initiation of the study, sub-samples of sediment were collected for each sampled site and submitted to CAS for chemical analyses (see section 5.3.9). Furthermore, at the end of the study, samples of sediments were also collected from each exposure chamber and submitted for further analytical testing (see section 5.3.9). In addition, confirmatory analytical testing of reference and control sediments was also completed prior to study initiation.

5.3.7 Chemical Analysis and Water Quality

As required by the design (see Appendix D, Supplemental Materials Order 3), temperature, pH, dissolved oxygen (DO), and conductivity were monitored daily using appropriate YSI electrodes (YSI Inc., Yellow Springs, OH, USA), while alkalinity, inorganic nitrogen, such as ammonia and nitrate, and hardness were monitored weekly using LaMotte Company colorimetric and titration test kits (Chestertown, MD, USA). In addition, pore water (at

2.5 cm below the sediment surface), sediment-water interface, and overlying water samples were collected weekly and submitted to CAS for chemical analyses.

All water samples were analyzed for target analyte list (TAL) metals, major cations/anions, alkalinity, hardness, and organic carbon (dissolved and total fractions). TAL metals (dissolved and total fractions) included: aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), Cd, chromium (Cr), cobalt (Co), Cu, iron (Fe), Pb, manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), thallium (Tl), vanadium (V), and Zn. Major cations/anions as defined for this study include: calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), sulfate (SO4), chloride (Cl), and fluoride (F). All water samples, including pore water, sediment-water interface, and overlying water, were extracted using acid-cleaned and nanopure water rinsed HDPE syringes. With the exception of dissolved fractions, extracted samples were directly discharged into pre-preserved sampling containers (see Appendix D, Supplemental Materials Table D9 for list of preservatives), and transported at 4 °C to CAS for chemical analysis. Samples in which analyses required the dissolved fraction were filtered through 0.45-µm polyethersulfone filters before being transferred into pre-preserved sampling containers. A summary of analytical methods and associated detection limits employed by CAS for water samples in the present study is provided in Appendix D, Supplemental Materials Table D4.

Total concentrations of TAL metals, acid volatile sulfide (AVS) and simultaneously extracted metals (SEM), total organic carbon (TOC), polychlorinated biphenyls (PCBs), organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), pH, and grain size were

determined for all samples of sediment. Organochlorine pesticides included dichlorodiphenyltrichloroethane DDT), dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyldichloroethane (DDD). A summary of analytical methods and associated detection limits for sediment/substratum samples is provided in Appendix D, Supplemental Materials Table D3.

5.3.8 Validation Assessment- Overall Data Quality

Environmental Standards Inc. (ESI; Valley Forge, PA, USA) performed an independent quality assurance and data validation review of the results produced by CAS. The review was performed in accordance with requirements specified by US EPA guidance documents (EPA 1992, 1999, 2004, 2009). Data were examined to determine usability of the analytical results and compliance relative to requirements specified above and the analytical methods. In addition, deliverables were evaluated for completeness and accuracy. Most analytical data were useable (> 88 %), with qualifications presented in data validation reports and summarized in Appendix D2 Supplemental Materials. Only useable data were included herein. There were instances where analytes were considered "not-detected" because they were detected at concentrations equivalent to that in the associated field blanks. For these samples, concentrations were reported as the limit of quantification (EPA 1992, 2009). In cases where measured values were 10x ≥ the associated blank, measured values were reported directly without correction. An EPA Quality Assurance/Quality Control (QA/QC) chemist reviewed the draft data and data validation reports, and EPA approved the data for public use.

During routine cleaning operations of exposure chambers UMF-D (Day 22) and CTRL-D (Day 23), a significant number of sturgeon fry were lost. Given that these events occurred well beyond the 48-hr permissible re-seeding window, these exposure chambers could no longer be used for biological measurements, such as survival and growth. Nevertheless, in an attempt to salvage information from these exposure chambers, they were converted into and designated as "chemistry-only" replicates (see section 5.3.3).

5.3.9 Statistics

Analytical data were categorized based upon sample type, including overlying water, sediment-water interface, and pore water, treatment type, such as sediment source at LALL, GE, DE, NP, LD, UMF, or LMF, tank replicate, and measurement type, including acid-extractable and dissolved metal. These categories allowed for explicit and systematic processing of data to quantify and evaluate a wide-range of exposure conditions throughout the duration of the study.

Concentrations were summarized on the basis of sediment source, replicate tank, and depth within exposure chambers. In addition, techniques for collecting samples of water were factored into the analysis. As noted in sections 5.3.5 and 5.3.6, as many as three distinct sampling techniques, such as suction, peepers, and DGT probes, were employed to characterize sediment-water interface and pore water samples. Data for each of these sampling techniques were used to quantify concentrations of various analytes within exposure chambers.

In some instances, concentrations of metals were less than the limit of quantification (LOQ) and thus, resulted in non-detectable concentrations. Given the relatively large number of non-detectable concentrations observed during the present study, summary statistics and

statistical comparisons and correlations were determined using maximum likelihood estimate (MLE) procedures. MLE procedures consider the presence of non-detectable concentrations when estimating parameters such as the mean, median, and variance for a given dataset. Procedures such as MLE provide better estimates of statistics for censored data, for samples for which the concentration is less than the limit of detection than simple "blind" calculations that treat BDLs as detected measurements or 'fabricating' values with the use of substitution methods, such as one-half the value of the detection limit (Helsel 1990). A detailed description and reasoning for use of the MLE procedure employed in the present study is provided in Appendix D1, Supplemental Materials. Conformation of distributions of data to approximate the normal probability function and equal variances was assessed by use of box and probability plots. If parametric assumptions were met, statistical comparisons of concentrations of metals within the different matrices of exposure chambers containing site sediments versus reference sediments were calculated using analysis of variance (ANOVA) and Dunnett's equivalents following MLE procedures. If parametric assumptions were violated, a Wilcoxon Score Test was performed following MLE procedures. When appropriate, statistical significance was adjusted with a Bonferroni correction factor. In addition, to investigate relationships between sampling techniques within a matrix, and concentrations of metal in sediment to concentrations in pore water and overlying water, linear regression and correlation coefficients were used following MLE procedures. Linear regression analyses were performed on measurements obtained from active sampling techniques, such as acid-extractable metals in whole sediments and sampling of matrices through suction. All data analysis procedures were conducted with R version 2.9.1 (R Development Core Team 2009).

5.4 Results and Discussion

5.4.1 Characterization of Sediments

Despite the relatively large sampling area, the presence of coarse-grained substrata, such as gravels, cobbles, and boulders, and an armored riverbed made collecting sufficiently large volumes of sediment difficult in some locations. Sediments were collected from NP, LD, UMF, LMF, LALL and GE, with volumes at LD, NP, and LMF being less than the targeted 600L (Table 5.2). Insufficient volumes of sediment were retrieved from CB, DE, and BBE. Given the difficulty in collecting sufficient volumes of site sediments, substrata collected above the water line from the gravel bar at DE were incorporated into this study, as directed by EPA.

With locations where sediment was collected targeted in areas north of RM-703 that contain white sturgeon spawning- and/or nursing-grounds, it is reasonable that predominant sediment grain sizes collected and evaluated for the present study were sand-sized particles, having diameters ranging from very coarse (1 < 2.0 mm) to very fine (62.5 < 125 μm) sands. The mean grain size distribution of site sediments was approximately 0.5 % gravels, 97.3 % sands, and 1.9 % silts/clays (Table 5.3). Reference sediments were slightly coarser with a mean grain size distribution of 20.9 % gravels, 76.5 % sands, and 1.4 % silts/clays.

Since the primary focus of this study was to assess toxicity of early life stages of white sturgeon, under laboratory controlled conditions, to a gradient of COPC associated with sediments, with a primary focus on those COPC commonly associated with granulated slag, the target metals were Cd, Cu, Pb and Zn. The analyses presented here focus on these four metals.

Table 5.2. Volumes of sediment collected for determining toxicity of sediments to white sturgeon in the Columbia River sediment toxicity study.

Sample Area Description	Sample Location	River Mile	Volume (gal)
References			
Lower Arrow Lakes	LALL	788	50
Genelle	GE	766	50
Birchbank	BBE	764	0
Site Sediments/Substrates			
Deadman's Eddy	DME-01	737	0
	DME-02		0
	DME-02		0
Gravel Bar at DME	DE	738	50
Northport	NP-01	735	0
	NP-02		0
	NP-03		15
China Bend	CB-01	723	0
	CB-02		0
	CB-03		0
Little Dalles	LD-01	729	50
	LD-02		0
	LD-03		0
Upper Marcus Flats	UMF-01	706	50
	UMF-02		50
	UMF-03		50
Lower Marcus Flats	LMF-01	704	50
	LMF-02		25
	LMF-03		5

Notes:
BBE - Birchbank Eddy
CB - China Bend
CTRL - artificial substrate control
DE - substrates as collected above the water line from the gravel bar at DME
DME - Deadman's Eddy
GE - Genelle Eddy
H20 - water-only control
LALL - Lower Arrow Lakes
LD - Little Dalles
LMF - Lower Marcus Flats
NP - Northport
UMF - Upper Marcus Flats

However, data for all COPC in whole sediment are presented in Appendix D3, Supplemental Materials. Sediment concentrations of Cd, Cu, Pb and Zn were significantly greater ($p < 0.01$) in all site sediment samples than in reference sediments (Appendix D, Supplemental Materials Table D5).

Concentrations of metals spanned the spectrum of concentrations observed to date within the site, and often exceeded the 90th centile of previously reported data (Figure 5.1; EPA 2006 a, b). Similarly, concentrations within reference sediments were lesser, often less than the 10th centile of site sediments. Based on these results, sediments evaluated for this study appear representative and consistent of the range of concentrations observed with site sediments.

Table 5.3. Summary of grain size distribution for sediments evaluated in exposure chambers for white sturgeon sediment toxicity tests.

			Exposure Chamber Treatment						
Aggregate Name	Grain Size (Range)	Units	DE	NP	LD	UMF	LMF	GE	LALL
Cobbles	64 to < 256	mm	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Very Coarse Gravel	32 to < 64	mm	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Coarse Gravel	16 to < 32	mm	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.8%
Medium Gravel	8 to < 16	mm	0.0%	0.0%	0.0%	0.0%	0.0%	5.2%	9.0%
Fine Gravel	4 to < 8	mm	0.2%	0.7%	0.0%	0.0%	0.0%	6.7%	5.0%
Very Fine Gravel	2 to < 4	mm	0.8%	0.1%	0.0%	0.4%	0.3%	6.8%	8.3%
Very Coarse Sand	1 to < 2	mm	2.0%	0.3%	0.5%	2.6%	3.5%	12.9%	13.7%
Coarse Sand	0.5 to < 1	mm	42.4%	1.0%	33.1%	38.8%	44.7%	37.0%	20.3%
Medium Sand	0.25 to < 0.5	mm	47.6%	50.0%	59.7%	49.9%	41.3%	23.6%	25.0%
Fine Sand	125 to < 250	µm	5.4%	39.3%	5.2%	5.3%	3.1%	3.7%	13.1%
Very Fine Sand	62.5 to < 125	µm	1.4%	5.5%	1.3%	1.1%	1.6%	1.6%	2.1%
Silt	< 62.5	µm	0.5%	1.6%	0.5%	0.9%	2.3%	0.6%	1.2%
Clay	< 62.5	µm	0.3%	1.0%	0.5%	0.3%	1.6%	0.5%	0.6%
Overall General Grain Size Distribution									
		Gravels	1.0%	0.8%	0.0%	0.4%	0.3%	18.6%	23.1%
		Sands	98.7%	96.2%	99.8%	97.6%	94.1%	78.8%	74.2%
		Clay/Silt	0.8%	2.6%	1.0%	1.2%	3.9%	1.1%	1.8%

Notes:
DE - substrates as collected above the water line from the gravel bar at DME
GE - Genelle Eddy
LALL - Lower Arrow Lakes
LD - Little Dalles
LMF - Lower Marcus Flats
NP - Northport
UMF - Upper Marcus Flats

5.4.2 Characterization Water Samples

5.4.2.1 Major Cation/Anion Water Quality Conditions

Concentrations for major cations/anions, including calcium (Ca^{++}), potassium (K^{+}), sodium (Na^{+}), and sulfate (SO_4^{-2}) within overlying water and pore water (at 2.5 cm depth) were

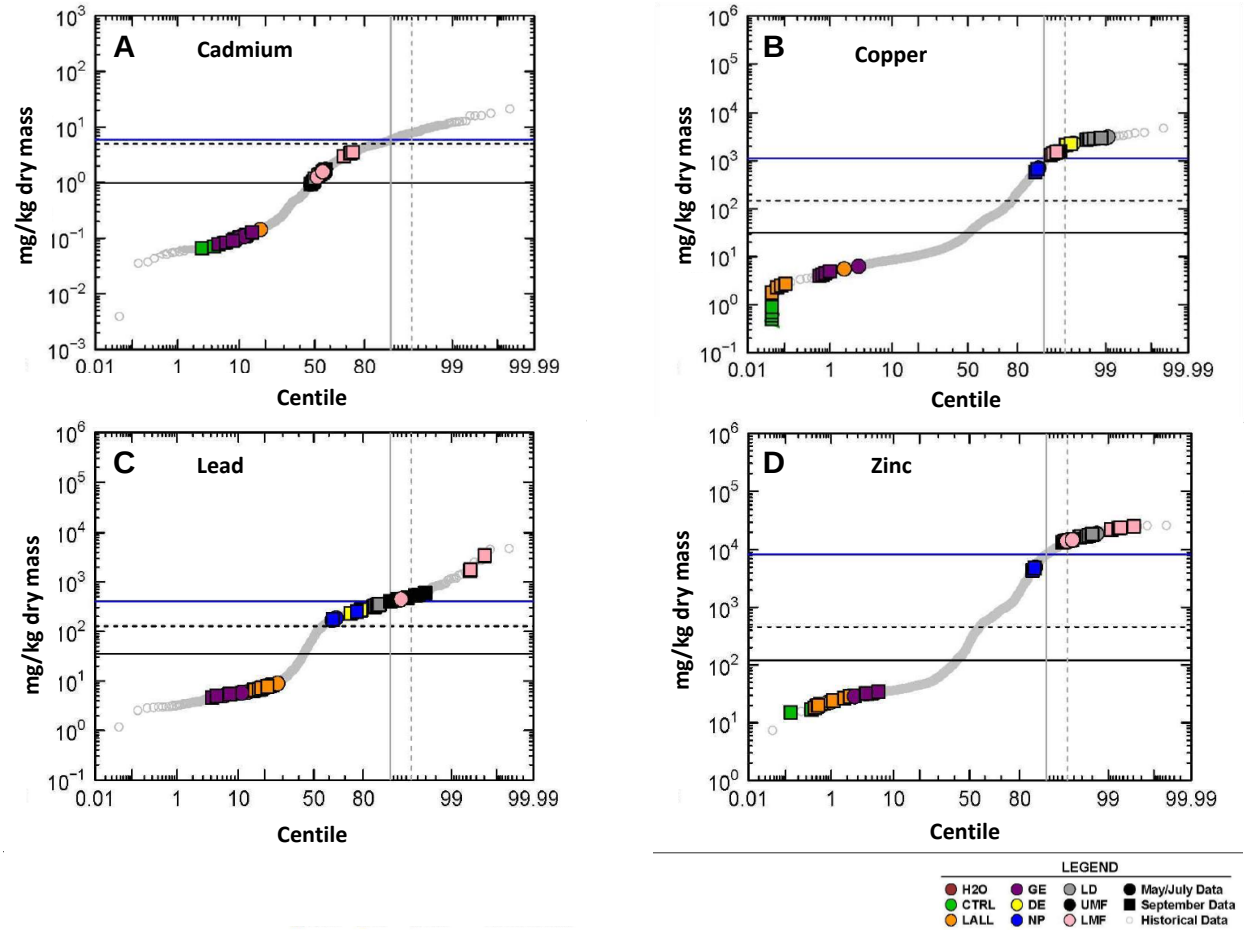

Figure 5.1. Total concentrations of acid-extractable metals of concern, cadmium (A), copper (B), lead (C), and zinc (D) in sediment samples evaluated within the sturgeon sediment toxicity tests. The 90th centile of the distribution is designated with a solid horizontal blue line. A dashed line and a solid black line are used to identify the probably effect concentration and threshold effect concentration, respectively, as defined by MacDonald et al. (2000) and are utilized and discussed in a parallel article and Chapter to characterize risk (Vardy et al. 2014c; Chapter 6).

consistent between treatments and for the duration of the study (Appendix D, Supplemental Material Table D8). Unlike major cations/anions, concentrations of DOC were more variable among treatments (e.g., LALL and DE) and greater within pore waters than overlying waters. A significant number of measured concentrations of DOC were qualified as estimated due to field

duplicate imprecisions. As a result, summary statistics for reported DOC concentrations were corrected for these imprecisions by factoring in any reported blank contamination (see Chapter 6 section 6.3.4.1 for details).

Concentrations of major cations/anions in water collected from the sediment-water interface measured by use of suction (pipette) and peeper techniques were comparable, with concentrations calculated by use of DGT probes being different (Appendix D, Supplemental Material Table D8). Given that DGT probes used in the present study were specifically designed and deployed to measure the flux of the four primary metals of interest (Cu, Cd, Pb, and Zn), calculated concentrations of major cations for the DGTs was likely due to saturation of the resin in the DGTs. Because diffusion coefficients are comparable in magnitude, fluxes of the cations comprising the hardness are on the order of 2,000 to 20,000 times greater than typical trace metal fluxes, the potential existed for the resin to become saturated over a time scale of hours rather than days (Zhang and Davison 1995a, 1999). Data for pore water quality at the 1 cm depth was limited because samples were collected via peepers and DGT probes only.

5.4.2.2 Dissolved Concentrations of Target Metals

5.4.2.2.1 Copper

There were significant differences ($p < 0.01$) in concentrations of dissolved Cu between exposure chambers containing site sediments versus reference sediments (Figure 5.2; Appendix D, Supplemental Materials Table D6). Dissolved concentrations of Cu in negative controls (H_2O and CTRL) and reference sediment (LALL and GE) exposure chambers were consistently lesser for the duration of the study with estimated median concentrations ≤ 1 µg/L for all matrices,

including overlying water, sediment-water interface, and pore water. In comparison, concentrations of Cu measured in samples containing UCR site sediments were significantly greater ($p \leq 0.001$) in all matrices compared to those of reference sediments, with the exception of a few measurements from passive sampling devices in DE and LMF sediment, and active sampling at the sediment-water interface in NP sediments (Appendix D, Supplemental Materials Table D6). The greatest concentrations of Cu were observed in pore water collected at a depth of 2.5 cm. In exposure chambers containing DE, LD, and UMF sediments median concentrations of 30, 20, and 10 μg Cu/L, respectively were measured in pore waters (Figure 5.2). In contrast, estimated median concentrations of Cu in pore water at 2.5 cm for exposure chambers containing sediments from NP and LMF approached 3 and 1 μg/L, respectively. Concentrations of Cu in pore water collected at a depth of 1 cm were less than those samples collected at 2.5 cm for exposure chambers containing DE, LD, and UMF sediments, with estimated median values < 4 μg/L. Concentrations of Cu were comparable between pore water depths for exposure chambers containing NP and LMF sediments. In overlying water and at the sediment-water interface, estimated median concentrations of Cu in samples from exposure chambers containing site sediments (0.8 – 2 μg/L) were equal to or slightly greater than the estimated median concentrations of Cu in control and reference sediments (< 1 μg/L).

All sampling devices used to extract samples from the sediment-water interface or from pore water were in agreement for concentrations of Cu (Figure 5.2). Concentrations of Cu measured in pore water at 1 cm below the sediment surface using peepers and DGT probes were significantly correlated ($p < 0.001$) and not statistically different ($p > 0.05$; Appendix D, Supplemental Materials Table D7). Similarly, concentrations of Cu measured at the sediment-

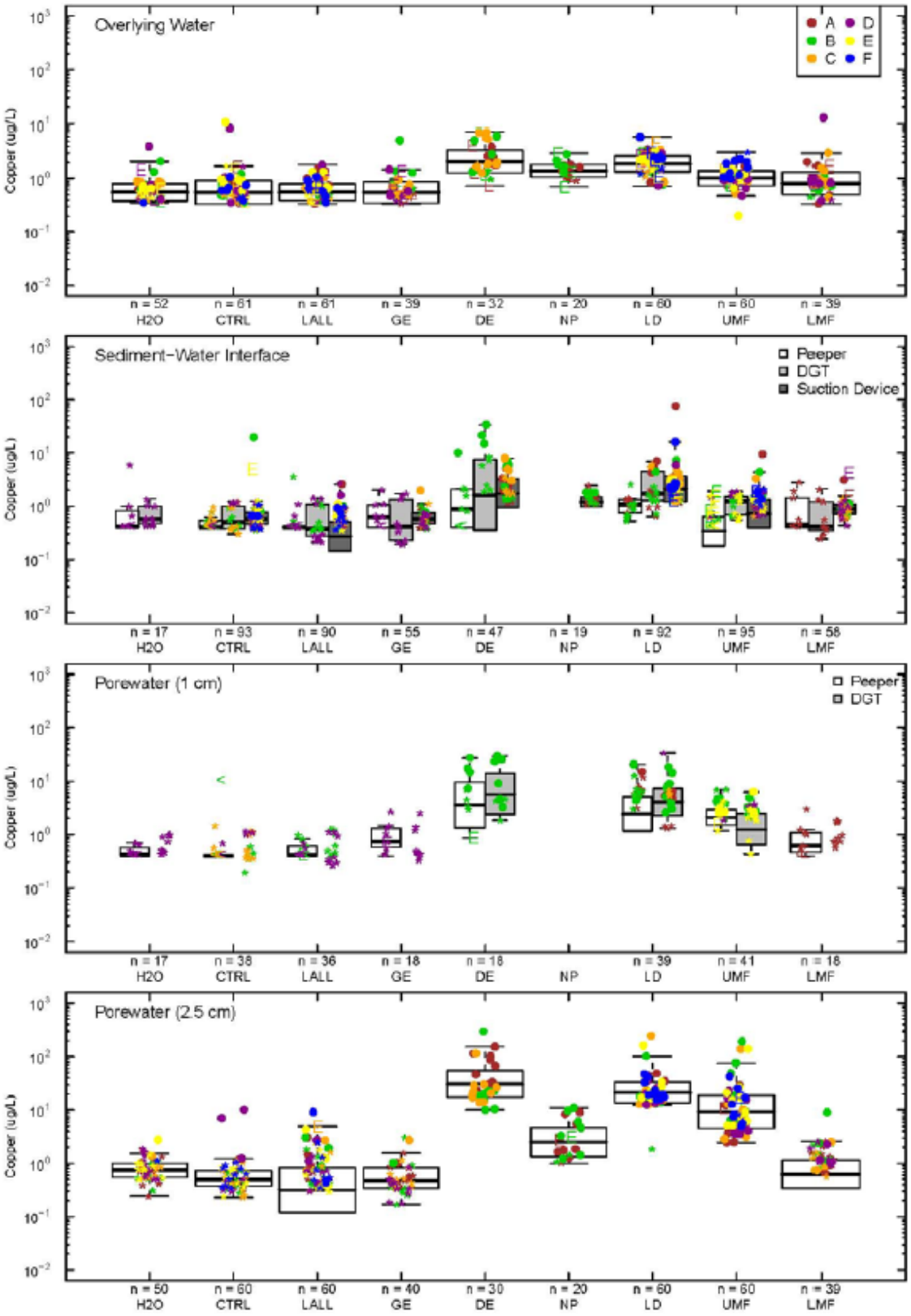
Overlying Water
A
B
C
D
E
F
Copper (ug/L)
n = 52 H2O
n = 61 CTRL
n = 61 LALL
n = 39 GE
n = 32 DE
n = 20 NP
n = 60 LD
n = 60 UMF
n = 39 LMF
Sediment-Water Interface
Peeper
DGT
Suction Device
n = 17 H2O
n = 93 CTRL
n = 90 LALL
n = 55 GE
n = 47 DE
n = 19 NP
n = 92 LD
n = 95 UMF
n = 58 LMF
Porewater (1 cm)
Peeper
DGT
n = 17 H2O
n = 38 CTRL
n = 36 LALL
n = 18 GE
n = 18 DE
NP
n = 39 LD
n = 41 UMF
n = 18 LMF
Porewater (2.5 cm)
n = 50 H2O
n = 60 CTRL
n = 60 LALL
n = 40 GE
n = 30 DE
n = 20 NP
n = 60 LD
n = 60 UMF
n = 39 LMF

Figure 5.2. Concentrations of dissolved copper as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Appendix D1, Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

water interface were significantly correlated ($p < 0.05$) among the three sampling techniques, including pipette suction, peeper, and DGT probes, and not statistically different ($p > 0.05$) between peeper and DGT techniques, and DGT and suction techniques (Appendix D, Supplemental Materials Table D7), with measurements from DGT probes being less consistent within sites. Differences between concentrations, achieved with any of the aforementioned sampling devices, appeared to be small and random, which suggests that any of these sampling methods could be used to characterize concentrations of Cu in pore water and water at the sediment-water interface.

Results of linear regression indicated that in all site sediment samples there was a significant positive relationship between concentrations of Cu in sediment to concentrations in pore water and overlying water. This indicated that acid-extractable concentrations of Cu in sediment were a reasonable predictor of concentrations of Cu in pore water and could prove to be

useful in assessing bioavailability and risk (DE r^2 = 0.85; NP r^2 = 0.82; LD r^2 = 0.92; UMF r^2 = 0.91; LMF r^2 = 0.77).

5.4.2.2.2 Zinc

Differences in median concentrations of Zn among all exposure chambers, regardless of sediment source, were relatively small (Figure 5.3). Estimated concentrations of Zn in overlying water in negative control (H_2O and CTRL) and reference sediment (LALL and GE) exposure chambers were < 6 µg/L. Concentrations of Zn in overlying water measured in exposure chambers containing site sediments had slightly greater concentrations with estimates ranging between 6 and 15 µg/L and were considered statistically different ($p < 0.001$) from GE reference sediment but not LALL reference sediment, with the exception of the LMF and LALL comparisons ($p \leq 0.001$; Appendix D, Supplemental Materials Table D6). Differences in concentrations of Zn at the sediment-water interface were lesser between negative controls, references, and site sediments. However, concentrations of Zn measured in water at the sediment-water interface with passive sampling devices in all site sediments were statistically greater ($p \leq 0.001$) from LALL reference sediments. Median concentrations of Zn in pore water (at 1 cm) from exposure chambers containing site sediments ranged between 3 and < 70 µg/L (Figure 5.3), and concentrations varied within sites depending on sampling technique, with the greatest concentrations measured in DGT probes. Regardless, median concentrations of Zn in pore water (at 1 cm) were slightly greater than those of the negative control and/or reference pore water (at 1 cm) concentrations (3 – 15 µg/L). All concentrations of Zn in pore water (at 1 cm) in site sediments were statistically greater ($p \leq 0.001$) than concentrations of Zn in pore water (at 1

cm) of one or more reference sediments (Appendix D, Supplemental Materials Table D6). Concentrations of Zn for pore waters collected at 2.5 cm fluctuated within sites and across all exposure chambers and ranged from 1 to 1000 μg/L. However, values were largely qualified, primarily due to contamination in blanks (Figure 5.3), and results should be interpreted with caution. Zn is ubiquitous in the environment and in the laboratory and the exact sources of contamination in the present study are unknown.

The different sampling methods used to quantify Zn in pore water or at the sediment-water interface often resulted in similar median measurements among techniques (Figure 5.3). Concentrations of Zn measured in pore water at 1 cm below the sediment surface using peepers and DGT probes were significantly correlated ($p < 0.001$) and not statistically different ($p > 0.05$; Appendix D, Supplemental Materials Table D7). At the sediment-water interface, only concentrations of Zn measured in peepers and DGT probes were significantly correlated ($p \leq 0.05$), but were not statistically similar ($p < 0.05$) with a tendency for measurements from peepers to be slightly less than from DGTs and suction devices. Overall, the sampling methods used to characterize concentrations of Zn in pore water appear to be more interchangeable than methods used to characterize concentrations of Zn at the sediment-water interface. Results of linear regression indicated that in all site sediments there was a significant positive relationship between concentrations of Zn in sediment to concentrations in pore water and overlying water. However, r^2 values were ≤ 0.50 in all site sediments, indicating that concentrations of Zn in sediment are a poor predictor of concentrations of Zn in pore water (DE $r^2 = 0.49$; NP $r^2 = 0.33$; LD $r^2 = 0.50$; UMF $r^2 = 0.40$; LMF $r^2 = 0.27$).

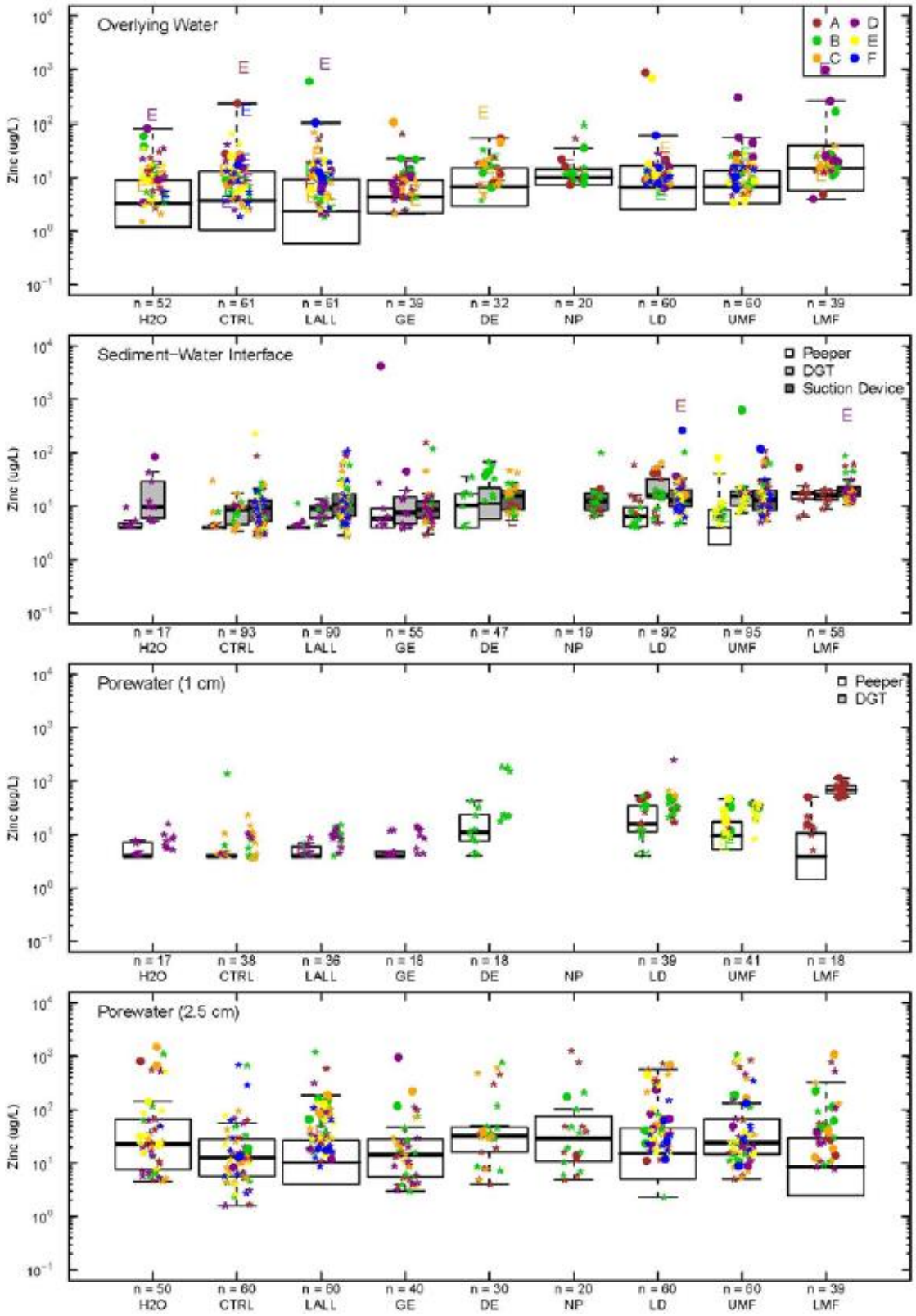
Overlying Water
A
B
C
D
E
F
Zinc (ug/L)
n = 52 H2O
n = 61 CTRL
n = 61 LALL
n = 39 GE
n = 32 DE
n = 20 NP
n = 60 LD
n = 60 UMF
n = 39 LMF
Sediment-Water Interface
Peeper
DGT
Suction Device
n = 17 H2O
n = 93 CTRL
n = 90 LALL
n = 55 GE
n = 47 DE
n = 19 NP
n = 92 LD
n = 95 UMF
n = 58 LMF
Porewater (1 cm)
Peeper
DGT
n = 17 H2O
n = 38 CTRL
n = 36 LALL
n = 18 GE
n = 18 DE
NP
n = 39 LD
n = 41 UMF
n = 18 LMF
Porewater (2.5 cm)
n = 50 H2O
n = 60 CTRL
n = 60 LALL
n = 40 GE
n = 30 DE
n = 20 NP
n = 60 LD
n = 60 UMF
n = 39 LMF

Figure 5.3. Concentrations of dissolved zinc as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Appendix D1, Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

5.4.2.2.3 Cadmium

Median concentrations of Cd within all exposure chambers, including site sediments, for all sample types, including overlying water, sediment-water interface, and pore water, and all sampling techniques were generally ≤ 0.1 μg/L (Figure 5.4). Concentrations of Cd in Pore water at 2.5 cm depth in exposure chambers with site sediments were significantly greater ($p \leq 0.001$) than exposure chambers with a reference sediment (Appendix D, Supplemental Materials Table D6). Specifically, median concentrations of Cd (0.2, 0.07, and 0.15 μg/L) in exposure chambers with DE, NP, and UMF sediments, respectively, were significantly greater than those of the negative control and reference sediment exposure chambers (0.02 – 0.04 μg Cd/L). Based on measurements from active sampling devices, concentrations of Cd in overlying water, pore water, and at the sediment-water interface in exposure chambers with DE and UMF sediments were significantly greater than exposure chambers with a reference sediment. In contrast, there

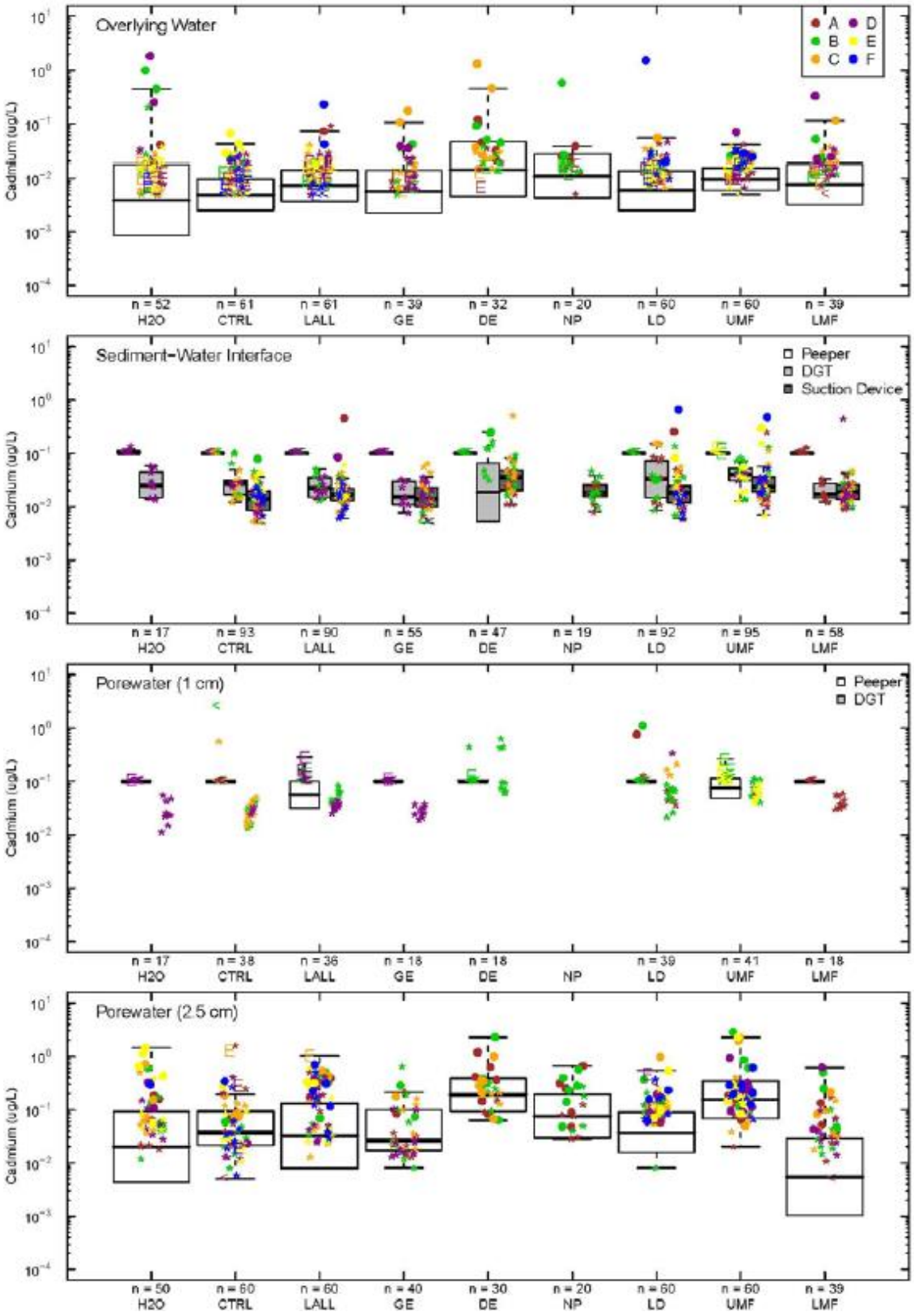
Overlying Water
A
B
C
D
E
F
Cadmium (ug/L)
n = 52 H2O
n = 61 CTRL
n = 61 LALL
n = 39 GE
n = 32 DE
n = 20 NP
n = 60 LD
n = 60 UMF
n = 39 LMF
Sediment−Water Interface
Peeper
DGT
Suction Device
n = 17 H2O
n = 93 CTRL
n = 90 LALL
n = 55 GE
n = 47 DE
n = 19 NP
n = 92 LD
n = 95 UMF
n = 58 LMF
Porewater (1 cm)
Peeper
DGT
n = 17 H2O
n = 38 CTRL
n = 36 LALL
n = 18 GE
n = 18 DE
NP
n = 39 LD
n = 41 UMF
n = 18 LMF
Porewater (2.5 cm)
n = 50 H2O
n = 60 CTRL
n = 60 LALL
n = 40 GE
n = 30 DE
n = 20 NP
n = 60 LD
n = 60 UMF
n = 39 LMF

Figure 5.4. Concentrations of dissolved cadmium as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Appendix D1, Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

were no statistical differences in concentrations of Cd between all site sediments and reference sediments in pore water or at the sediment-water interface based on peeper measurements.

Of the three sampling devices used to measure sediment-water interface samples, measurements with DGTs and suction devices demonstrated relatively good agreement for concentrations of Cd and were significantly correlated ($p \leq 0.05$), although statistically different, while measurements made with peepers were consistently greater (Figure 5.4, Appendix D Supplemental Material Table D7). Likewise, measurements in pore water made with peepers at 1 cm depth demonstrated poor correlation and were consistently greater for concentrations of Cd than measurements of pore water with DGTs at the same depth. Based on these results, DGTs, peepers, and suction devices do not appear to be interchangeable methods for measuring concentrations of Cd.

Results of linear regression indicated that in all site sediment samples there was a significant positive relationship between concentrations of Cd in sediment to concentrations in pore water and overlying water. Only in certain site sediment samples, however, were concentrations of Cd in sediment moderate predictors of concentrations of Cd in pore water (DE $r^2 = 0.65$; NP $r^2 = 0.71$; LD $r^2 = 0.68$; UMF $r^2 = 0.63$; LMF $r^2 = 0.50$).

5.4.2.2.4 Lead

Median concentrations of Pb within all exposure chambers, including site sediments, for all sample types, such as overlying water, sediment-water interface, and pore water, and all sampling techniques were generally < 1.0 μg/L (Figure 5.5). Differences in estimated median concentrations of Pb within the different matrices were relatively small between negative controls, reference sediments, and site sediments. However, concentrations of Pb in all site sediments were significantly greater ($p \leq 0.001$) in overlying water and at the sediment-water interface than in reference sediment, based on measurements from active sampling techniques (Appendix D, Supplemental Materials Table D6). Also, concentrations of Pb in pore water measured in samples collected by active sampling (suction at 2.5 cm depth) in exposure chambers containing DE, UMF, and LMF sediments were significantly greater than those of reference sediments. In contrast, there were no significant differences in concentrations of Pb in pore water or at the sediment-water interface in DE, LD, or UMF sediments compared to reference sediments, based on passive sampling techniques. There were no significant correlations among concentrations of Pb among different sampling techniques utilized in pore water or sediment-water interface measurements. The different sampling techniques within

individual exposure chambers measured different concentrations of Pb at the sediment-water interface, with a tendency towards elevated concentrations of Pb in samples from peepers in comparison to DGTs and suction devices. A similar tendency towards greater concentrations of Pb measured in pore water at 1 cm with peepers versus DGTs was also observed. Based on these results, DGTs, peepers, and suction devices do not appear to be interchangeable methods for measuring concentrations of Pb. Results of linear regression indicated that in all site sediment samples there was a significant positive relationship between concentrations of Pb in sediment to concentrations in pore water and overlying water. Concentrations of Pb in sediment were moderate predictors of concentrations of Pb in pore water (DE $r^2 = 0.65$; NP $r^2 = 0.71$; LD $r^2 = 0.58$; UMF $r^2 = 0.70$; LMF $r^2 = 0.64$).

5.4.2.2.5 Other Metals

There were no major differences in concentrations of the additional metals analyzed in pore water, overlying water, or at the sediment-water interface, between exposure chambers containing site sediments and reference sediments, except for antimony (Appendix D3, Supplemental Materials). Estimated median concentrations of antimony in site sediment treatment groups were slightly elevated in overlying water and at the sediment-water interface, whereas concentrations in pore water differed significantly ($p \leq 0.001$) in exposure chambers containing site sediments (0.1 – 100 μg/L) compared to reference sediments (< 0.5 μg/L).

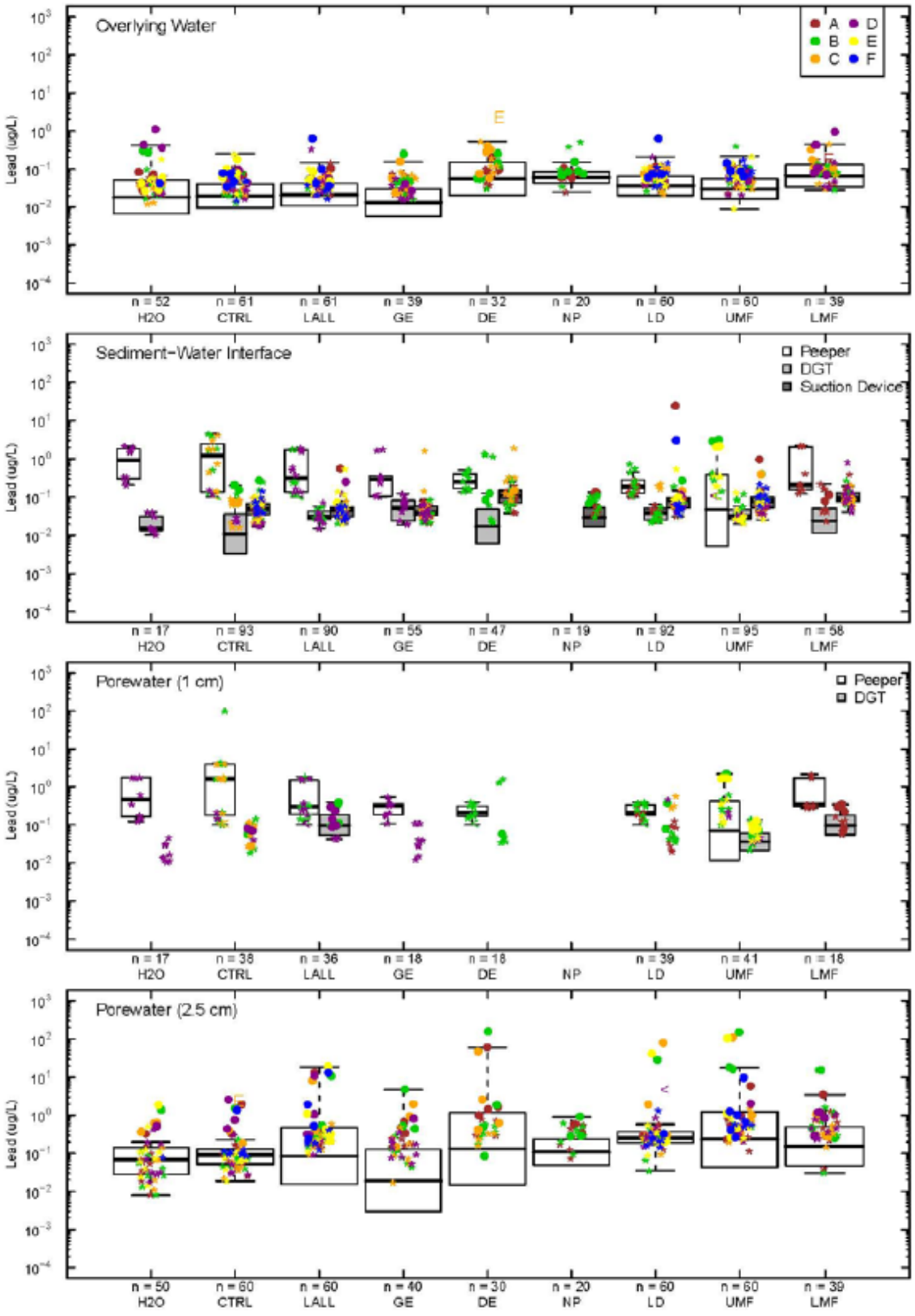
Overlying Water
A
B
C
D
E
F
E
Lead (ug/L)
n = 52 H2O
n = 61 CTRL
n = 61 LALL
n = 39 GE
n = 32 DE
n = 20 NP
n = 60 LD
n = 60 UMF
n = 39 LMF
Sediment-Water Interface
Peeper
DGT
Suction Device
n = 17 H2O
n = 93 CTRL
n = 90 LALL
n = 55 GE
n = 47 DE
n = 19 NP
n = 92 LD
n = 95 UMF
n = 58 LMF
Porewater (1 cm)
Peeper
DGT
n = 17 H2O
n = 38 CTRL
n = 36 LALL
n = 18 GE
n = 18 DE
NP
n = 39 LD
n = 41 UMF
n = 18 LMF
Porewater (2.5 cm)
n = 50 H2O
n = 60 CTRL
n = 60 LALL
n = 40 GE
n = 30 DE
n = 20 NP
n = 60 LD
n = 60 UMF
n = 39 LMF

Figure 5.5. Concentrations of dissolved lead as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Appendix D1, Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

5.4.3 Comparisons of Sampling Techniques

There were differences in measured concentrations of metal among sampling techniques within a matrix for most of the metals analyzed. Considering all the TAL metals, concentrations of metals from DGTs and suction devices tended to be similar at the sediment-water interface whereas measurements from peepers were often greater in comparison. Similarly, in pore water at 1 cm depth, concentrations of metals in water in peepers were often greater than those estimated by DGTs. This trend could, in part, be due to the fact that concentrations in peepers represent metals averaged over time, once equilibrium has been established with the surrounding matrix, whereas suction devices measure point source concentrations of metals. Alternatively, assuming that the metal that is removed from a matrix is rapidly re-supplied from the solid phase in sediment, DGTs characterize fluxes of metals and provide a measurement of average concentrations of metals during deployment time within a matrix (Zhang et al. 2001). Previous studies have investigated issues with re-supply and have found that in cases where it is

insufficient, DGT measurements can result in calculated concentrations of metals that are significantly less than concentrations of metals measured in pore water using other, more traditional techniques, such as core sampling and centrifugation of sediments (Zhang et al. 1995b; Fones et al. 2001). For certain metals in the present study, however, such as Cu, Zn, and Al, concentrations of metals in water at the sediment-water interface and pore water at 1 cm depth were relatively similar between these two methods of sampling.

Use of airstones for extraction of pore water has several advantages over peepers and DGTs, including the ability to extract relatively large sample volumes that in turn allow for a greater suite of chemical analyses. In addition, airstones can be used for more than one sampling event without having to disturb the sediment. Peepers and DGTs, however, can sample more than one matrix at a time and can be used in the laboratory or in the field.

5.5 Conclusions

Sediments investigated during the present study covered a range of concentrations of targeted metals that were representative and consistent of the extent of concentrations observed with UCR site sediments, and captured the upper concentration range of previously reported data. In the present study, acid-extractable concentrations of Cu, Cd, Zn, and Pb in whole samples of site sediments were significantly greater than those in reference sediments. Of the four primary metals of concern, Cu, Cd, Pb, and Zn, concentrations of Cu, primarily in pore water, were greatest in exposure chambers containing site sediments compared to those in reference sediments. Concentrations of Cu and Zn were significantly greater in matrices of site sediments compared to reference sediments more often than Cd and Pb. Active and passive

sampling techniques resulted in similar estimates of concentrations of Cu in pore water and at the sediment-water interface, and comparable measurements for concentrations of Zn. In contrast, sampling techniques resulted in dissimilar measurements for concentrations of of Pb and Cd, with a tendency towards greater concentrations in peepers compared to DGTs in both pore water and at the sediment-water interface. Discrepancies in measurements of concentrations of metals between peepers and DGTs were a common trend for a number of the non-target metals analyzed in the present study. At the sediment-water interface, however, DGTs and active sampling through suction often resulted in relatively similar measurements for many of the metals.

Bioavailability of metals is a key factor to assessing risk of exposure to metals associated with sediment. In general, concentrations of dissolved metals in pore water are considered to be the most representative of the fraction of metal bioavailable to sediment-dwelling organisms. With contaminated sediments, there is a concern that metals might leach out of sediments into pore water at concentrations sufficient to have adverse effects on organisms associated with the sediments. When considering effects of metals associated with sediments on benthic dwelling fish such as sturgeon, matrices other than pore water might be a more applicable route of exposure. Therefore, the present study characterized concentrations of metals in various matrices associated with sediment. Concentrations of metals in sediment often resulted in significant concentrations of metals in pore water and overlying water; although results varied depending on the sampling technique employed. When active sampling through suction was considered, linear regression was a relatively effective means of characterizing the movement of metal in sediment to pore water and overlying water for certain metals such as Cu. However, the present study demonstrated that measured concentrations of dissolved metal in matrices associated with

sediment can differ depending on the choice of sampling method, matrix, and analyte. Differences in estimated concentrations of metals among the methods applied highlights the difficulty in assessing the true risk of exposure to metals associated with sediments. To minimize uncertainty, concentrations of metals could be measured in bodies of organisms, but this is difficult under field conditions.

Regardless of these differences among methods of sampling, concentrations of Cu, Cd, Pb, and Zn calculated from any of the techniques employed to sample water at the sediment-water interface or in overlying water in the present study were less than the EPA national criteria of 6.0 µg/L, 0.174 µg/L, 1.46 µg/L, and 78 µg/L, respectively, and criteria for the state of Washington for the protection of aquatic life of 7.4 µg/L, 1.51 µg/L, 1.46 µg/L, and 69 µg/L, respectively. These criteria do not take into account the bioavailable fraction of metals, except for EPA criteria for Cu that utilizes the Biotic Ligand Model. Therefore, when considering pore water, interpretations are more difficult since water quality characteristics, such as hardness and DOC, were more variable among sediments and affect site-specific water quality standards. In addition, questions as to whether or not benthic fish such sturgeon are exposed to metals associated with pore water become important. A more appropriate comparison would be concentrations corrected for chemical activity, and binding to inorganic and organic ligands by use of the Biotic Ligand Model. The analytical data reported herein are utilized in Chapter 6 and a parallel article to characterize risk, and in turn, compare predictions to the biological results from exposure of early life stage white sturgeon to metals in sediments of the Upper Columbia River (Vardy et al. 2014c).

CHAPTER 6

As previously stated, Upper Columbia River sediment toxicity to early life stages of white sturgeon is of concern. To investigate potential effects of metals associated with sediment, a chronic exposure study was conducted that characterized bioavailability of metals in Upper Columbia River sediment while comparing survival and growth of white sturgeon reared on site sediments versus reference sediments. The analytical findings and assessment of metal bioavailability in sediments were the subject of Chapter 5 and Vardy et al. (2014b), whereas the present Chapter and Vardy et al. (2014c) characterized risk from exposure to metals associated with sediments, and presents the physiological and biological responses of early life stage white sturgeon following sediment exposure. The following article is intended for publication: Vardy D, Doering J, Ryan A, Santore R, Hecker M, Giesy J. Manuscript in preparation. *Toxicity assessment of metals associated with sediments from the Columbia River to early life stages of white sturgeon.* A description of co-authorship can be found in Appendix F.

6.0 TOXICITY OF METALS ASSOCIATED WITH SEDIMENTS FROM THE COLUMBIA RIVER TO EARLY LIFE STAGES OF WHITE STURGEON

6.1 Abstract

There are increasing concerns regarding declines of sturgeon populations worldwide. A number of issues such as habitat alteration, overfishing, genetic bottlenecks, and pollution have been hypothesized as contributing factors. For some North American rivers such as the Upper Columbia, it has been hypothesized that metals associated with sediments might be contributing to poor recruitment of white sturgeon. In order to assess the potential effects of sediment-borne metals to early life stages of white sturgeon, the present study employed a laboratory-based,

flow-through exposure system to characterize toxicity of Upper Columbia River (UCR) sediments to early life stage white sturgeon under chronic exposure conditions. Sediments were collected from areas of the UCR known to be white sturgeon spawning- and/or nursing-grounds and containing a gradient of concentrations of metals of primary interest, copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn). Concentrations of metals in bulk sediment, pore water, overlying water, and water at the sediment-water interface, were measured to characterize the potential to affect early life stages of white sturgeon. Survival and growth of early life stage white sturgeon over 60 days were related to concentrations of metals in sediments and water and compared to thresholds for effects given in the literature. Based on probable effect concentrations (PECs) and excess simultaneously extracted metals (SEMX), site sediments in the present study had potential to elicit adverse effects to sediment-dwelling organisms. Additional data collected within pore water and overlying water within exposure chambers were then analyzed using the Biotic Ligand Model (BLM) to allow for more explicit consideration of bioavailability of Cu, Cd, Pb and Zn to white sturgeon. Of the metals of primary concern, BLM predictions indicated that concentrations of Cu in pore water exceeded the threshold for effects for a limited number of site sediments and replicate exposure chambers. However, effects predicted for measured concentrations were not observed in survival or growth of white sturgeon exposed to site sediments in the experimental chambers. There were statistically significant differences in growth of white sturgeon; however, these differences were largely explained by fish density at termination and not associated with any of the measured chemical factors including concentrations of the four target metals. Of the methods used in the present study to characterize potential effects due to exposure to metals associated with UCR sediments, predictions of the BLM corresponded best with the observed results for white sturgeon.

6.2 Introduction

Populations of sturgeon are threatened throughout the world. Factors such as age to reproductive maturity make sturgeons particularly susceptible to alterations in the environment and their benthic lifestyle might result in exposure of all life stages to contaminants that are associated with sediments. Alteration of habitats, including pollution, has been hypothesized to be a contributing factor to their global decline (Birstein 1993; Gisbert and Williot 2002; Hu et al. 2009; Irvine et al. 2007; Luk'yanenko et al. 1999; Paragamian and Hansen 2008). Specifically, in some North American rivers it has been hypothesized that metals associated with sediments might be contributing to poor recruitment of white sturgeon (*Acipenser transmontanus*; Hilderbrand et al. 2013; Paragamian and Hansen 2008). One population of particular concern that has been experiencing poor annual recruitment for over forty years resides in the Upper Columbia River (UCR), between Grand Coulee Dam in the USA and Hugh L. Keenleyside Dam in southern BC, Canada (Hildebrand et al. 1999, 2013; Irvine et al. 2007). In 2006, this population was listed as endangered by the Canadian government (COSEWIC 2013) and it is suspected that without a successful remedial program this population might face extinction within the next half century (Hildebrand et al. 1999, 2013; Irvine et al. 2007). Although specific reasons for the observed decreases in the number of sturgeon are not fully understood, pollution has been hypothesized as one potential contributor to recruitment failure of white sturgeon in the UCR (Hildebrand et al. 2013).

Municipal and industrial sources of pollution in the UCR include discharges from, among others, municipal wastewater treatment plants, the pulp and paper industry, and metallurgical operations (Hildebrand et al. 2013). In Trail, BC, Canada, a metal smelter has been operational

for over one hundred years and historically released slag, a by-product of the refining process, into the river. Consequently, metals such as copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn), have been found at concentrations in sediments downstream of the facility that are greater than those in sediments from reference locations (Besser et al. 2008; Bortleson et al. 2001; EPA 2006 a,b). In 2006, a remedial investigation and feasibility study (RI/FS) was initiated in the UCR, under the oversight of the US EPA (www.ucr-rifs.com). One of the concerns to be addressed by the RI/FS was potential toxicity of contaminants associated with sediments to early life stages of white sturgeon, including metals. Early life stages of white sturgeon inhabit benthic habitats, on the surface of sediment or in interstitial space between stones, and at the yolksac fry stage tend to hide in refugia (Brannon et al. 1983, 1985; Gessner et al. 2009; McAdam 2011; Richmond and Kynard 1995; personal observation in the laboratory).

Previous studies have calculated thresholds for effects of aqueous concentrations of metals to white sturgeon and have found early life stages to be relatively more sensitive to effects of certain metals, such as Cu, than early life stages of other fishes (Little et al. 2012, Vardy et al. 2011, 2013, 2014a). Other studies have investigated the release of elements from contaminated sediments in the Columbia River into pore water, overlying water, and supernatants of aggressively tumbled slurries, and found that under certain conditions, there might be exposure to concentrations of metals sufficient to cause adverse effects (Paulson and Cox, 2007). Therefore, the current study employed an experimental design with controlled fluvial laboratory exposure settings comparable to conditions found within the UCR stretch of concern to assess toxicity of contaminants associated with sediments from the UCR to early life stages of white sturgeon.

Results from the present study, which are to be incorporated in the UCR RI/FS and used to supplement information in a baseline ecological risk assessment (BERA), are presented in two parts. Concentrations of metals in sediments from the UCR and an assessment of their bioavailability are the subject of Chapter 5 (i.e. Vardy et al. 2014b). The present article characterized effects of potential exposure to metals associated with UCR sediments, and presents the physiological and biological responses of early life stage white sturgeon following exposure.

Concentrations of metals in different matrices such as pore water, overlying water, and water at the sediment-water interface were sampled by use of various sampling techniques and quantified to assess bioavailability. Peepers (Doig and Liber 2009), diffusive gradients in thin films (DGTs; Davison and Zhang 1994), and active sampling/suction techniques were employed throughout the experiment to characterize exposures to metals and chemical parameters in the different matrices. Various risk assessment approaches, including probable effect concentrations (PECs), excess simultaneously extracted metals (SEMX), and the biotic ligand model (BLM) were used to characterize risk to white sturgeon.

6.3 Methods

6.3.1 Study Design

Exposure to and potential effects of UCR sediments to early life stages of white sturgeon were assessed by use of flow-through chambers within re-circulating systems at the University of Saskatchewan Aquatic Toxicology Research Facility (UofS ATRF), Saskatoon, SK, Canada. The experimental methods, including selection of sites from which sediments were collected, efforts,

and study design have been described previously (Chapter 5). Early life stages of white sturgeon were exposed to UCR sediments in the laboratory for 60 d beginning from 1 day post hatch (dph). Sediments were collected from areas of the UCR known to be white sturgeon spawning- and/or nursing-grounds (Howell and McLellan 2006; Weakland et al. 2011) and containing a gradient of concentrations of contaminants of potential concern (COPC) in sediments associated with granulated slag. Specifically, the primary COPCs included Cd, Cu, Pb, and Zn (EPA 2006 a, b). Sediments were collected at five locations in the UCR downstream of the metallurgical facility in Trail, BC, Canada: Deadman's Eddy (DE), Northport (NP), Little Dalles (LD), Upper Marcus Flats (UMF), and Lower Marcus Flats (LMF; Chapter 5 and Vardy et al. 2014b), collectively referred to as "site sediments". Sediments from two reference locations were collected from areas upstream of the metallurgical facility at Genelle (GE) and Lower Arrow Lakes (LALL; Chapter 5 and Vardy et al. 2014b). In addition, an artificial sediment (Rolf C. Hagen, Inc. Aquarium Substrate Item No. 12648) and a water only exposure were included as negative controls (termed CTRL and H_20, respectively).

Suction devices were used to collect water in overlying water, defined as within the top 15 cm of the water column, and at the sediment-water interface, defined as the boundary between sediment and the overlying water column within 1 cm above the sediment surface. In addition, peepers and DGTs were employed to simultaneously sample water at the sediment-water interface as well as in pore water, 1 cm below the sediment surface. At 2.5 cm below the sediment surface, ceramic air-stones (RENA Micro Bubbler 6-in. Mars Inc. Hackettstown, NJ, USA) were distributed along the length of each exposure chamber for additional pore water sampling (Vardy et al. 2014b; Appendix D, Supplemental Materials Order 5 and 6).

6.3.2 Fish Culture and Exposure

Fertilized white sturgeon eggs were obtained from the Kootenay Trout Hatchery, Fort Steele, BC, Canada, and transported to the UofS ATRF where they were incubated until hatch. Transportation and incubation procedures followed the methods described by Vardy et al. (2011). Prior to initiation of tests, and placement of fish into the exposure systems, periphyton was allowed to grow on substrata to provide a grazing environment for sturgeon transitioning to feeding. At approximately 7 dph, food was introduced to the exposure chambers to familiarize white sturgeon larvae with a food scent. Fish were fed a combination of nauplii of live brine shrimp (*Artemia salina)*, a semi-moist powder diet containing one part # 0 trout chow, three parts cyclopeze, two parts krill and one part tubifex, and as their primary diet, frozen bloodworms (Hagen, San Francisco Bay Brand, Edmonton, AB, Canada). Fish were fed *ad libitum* four to eight times throughout the day and into the evening. Rates of feeding were increased when larvae were transitioning to feeding and fish were fed throughout the night, since this has been shown to be a critical period for survival (Vardy et al. 2011).

Endpoints included survival and growth of white sturgeon reared on site sediments versus reference sediments. During the course of the study, exposure chambers were visually inspected twice daily. As part of daily inspections, exposure chambers were monitored for mortalities. If a larval sturgeon died, it was removed using a fish net disinfected with net soak (Jungle Laboratories, Cibolo, Texas, USA), blotted dry, and mass determined to the nearest 0.001 g, length measured with calipers to the nearest 0.01 mm, and preserved in 10 % formalin for a period of 24 hrs. At the end of the 24 hr preservation period, the formalin was replaced with 70 % ethanol for long-term storage. At the end of the study, all remaining fish were sampled from

respective exposure chambers. Surviving fish were captured using a disinfected fish net, euthanized with Tricaine®-S (MS-222), and measured as above. At the end of the study, fish were collected over a two-day period.

6.3.3 Risk Characterization

Concentrations of metals in sediment and the different matrices associated with sediments, such as pore water, overlying water, and the sediment water interface, were measured to characterize risk using different hazard assessment approaches. The full suite of chemical analyses conducted on sediment and water samples has been previously described (Chapter 5). To evaluate potential effects of concentrations of metals in site sediments, PECs, threshold effect concentrations, and mean probable effect concentration quotients (mPECQs) for metal mixtures were calculated following the methods outlined by MacDonald et al. (2000). In addition, acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) were used to define SEMX (the difference SEM – AVS), and carbon-normalized excess SEM (SEMX, organic carbon [OC]; SEMX, OC = SEMX/ fraction of organic carbon in sediment [fOC]; Ankley et al. 1996; EPA 2005). Based on these estimates of bioavailable metal, benthic organisms should be adequately protected in sediments if SEM does not exceed AVS (SEMX $\leq$ 0) when AVS $\geq$ 0.1 μmol/g. On the basis of SEMX, it has been shown that sediments with SEMX < 1.7 μmol/g pose low risk of adverse biological effects, whereas sediments with SEMX > 120 μmol/g might be expected to cause adverse biological effects (EPA 2005). For SEMX between 1.7 and 120 μmol/g, the potential for toxicity is uncertain. Sediments with lesser carbon-normalized SEMX (< 130 μmol/gOC) should pose little risk of adverse biological effects due to SEMs. For sediments with greater carbon-normalized (SEMX > 3000 μmol/gOC) adverse biological effects due to SEMs

might be expected. For sediments with intermediate carbon-normalized SEMX (> 130 μmol/gOC and < 3000 μmol/gOC) there is uncertainty about whether effects are expected (EPA 2005).

6.3.3.1 Application of Biotic Ligand Model

The biotic ligand model (BLM; Di Toro et al. 2001; Santore et al. 2001, 2002; HydroQual 2007) was used to characterize risk of exposure to metals associated with UCR sediments to early life stages of white sturgeon. Input files for application of the BLM were prepared using the analytical dataset from the present study (Chapter 5), with model simulations utilizing conservative survival-based parameter files as developed from previous studies with early life stages of white sturgeon (Vardy et al. 2011, 2013). Predicted effect concentrations resulting from the BLM for the primary metals of interest (Cd, Cu, Pb and Zn) were then evaluated against measured dissolved metal concentrations measured during the study. In a limited number of instances and where appropriate, assumptions and mean values were used to represent input data to the BLM model for a given sample within an exposure chamber. For instance, if concentrations of sulfate and chloride were unavailable within a given sample type as collected within an exposure chamber, the mean value based on other samples collected within that exposure chamber were used.

A significant number of measured dissolved organic carbon (DOC) concentrations were qualified as estimated due to field duplicate imprecision (Chapter 5 and Vardy et al. 2014b). Therefore, prior to being used and incorporated as a BLM input file, measured concentrations of DOC were “blank-corrected” to account for this imprecision. Specifically, given that the mean DOC concentration recorded in quality assurance and quality control (QA/QC) samples, such as

measurement blanks and laboratory controls (H_2O) was 1.93 mg/L, DOC concentrations for all exposure chambers and sample types were "blank-corrected" by subtracting 1.93 mg/L from the measured DOC concentration. To ensure that measured DOC concentrations were not "over-corrected" (e.g., a negative value), "blank-corrected" concentrations were not allowed to be less than the mean DOC concentration for UofS ATRF testing waters of 1.50 mg/L.

Survival predicted with the BLM was based on measured survival in white sturgeon toxicity tests (Vardy et al. 2011, 2014a). Calibration of these survival endpoints was accomplished by adjusting the critical accumulation of metal at the biotic ligand (BL). To provide the most conservative evaluation, when multiple measures of survival were available, calibration of the BLM was based on the most sensitive observed endpoint (LC_{20}). Using this approach, resulting critical accumulation values at the BL for Cd, Cu, Pb and Zn were 2.5 nmol/gw, 0.0042 nmol/gw, 0.028 nmol/gw, and 1.2 nmol/gw, respectively. Predictions from the BLM model were compared with observed concentrations for each metal of interest in overlying water, water from sediment-water interface, and pore water (1 and 2.5 cm). To aid this comparison, toxic units (TU) were calculated by dividing respective measured metal concentrations by the BLM-predicted effects concentration. For a given exposure chamber, replicate, and type of matrix, the geometric mean of measured concentrations was calculated or estimated by use of the maximum likelihood estimate (MLE) procedure; described previously (Vardy et al. 2014b), and used to calculate TUs. If more than 80 % of measured concentrations were qualified (e.g., being below detection limit [BDL] or estimated) the calculated geometric mean was concomitantly flagged as a "<" value. As detailed in Appendix D1, Supplemental

Materials, this approach provides an upper bound of the exposure concentration, because the actual value will be less than this estimate.

6.3.4 Survival and Growth of Early Life Stages of White Sturgeon

Survival and growth were analyzed to determine if white sturgeon were adversely affected by exposure to UCR sediments, relative to fish exposed to reference sediments. Survival was analyzed to provide estimates of cumulative survival of individual white sturgeon at the end of the study as well as during the course of exposure. Survival analysis considers and accounts for all fish introduced to exposure chambers, including those fish culled or "missing". Size data for fish surviving to test termination were used to quantify the effect of exposure to site sediments on fish size.

6.3.5 Survival Analyses

The Kaplan-Meier method was used to estimate survival during the course of the study. The Kaplan-Meier method allows for a transparent and consistent treatment of data for all exposure chambers, regardless of whether or not censoring due to lost fish occurred (see Section 6.4.3 for explanation of lost fish in the present study). The Kaplan-Meier method operates on the number of fish at risk at any given time point, and if a fish is removed for any reason (other than death), it is not counted as a mortality event, but rather, it decreases the number of fish at risk of dying at subsequent time points. Mathematically, the Kaplan-Meier method is a product limit function (Equation 6.1):

$$\hat{S}(t_{(j)}) = \prod_{i=1}^{j} \hat{\Pr}[T > t_{(i)} \mid T \geq t_{(i)}] \qquad \text{(Equation 6.1)}$$

Where:

$\hat{S}(t_{(j)})$ = estimated survival probability at time j,

I = index of multiplication,

T = survival time, and

t = time=point of interest.

In practice, $\hat{S}(t_{(j)})$ can be determined directly from the survival data (Equation 6.2):

$$\hat{S}(t_{(j)}) = \prod_{i=1}^{j} \left(\frac{n_i - d_i}{n_i} \right) \qquad \text{(Equation 6.2)}$$

Where:

d_i = number of deaths at time *I*,

n_i = number of organisms at risk of dying at time *i*.

The effect of censoring due to lost fish can be incorporated in Equation 6.2 by defining $n_i = n_{i-1} - c_{i-1} - d_{i-1}$, such that the number of organisms censored (*c*) or died (*d*) at the previous time, time *i-1*, are removed from the risk set of interest, at time *i*. The ability to consider censored observations in the survival probability function provides a direct means of accounting for effects of loss of individuals, while allowing lost fish to contribute to estimates of survival up to the time at which those fish were lost from the exposure chamber.

The primary objective of this study was to determine if survival and growth of white sturgeon were adversely affected when fish were reared on site sediments versus reference sediments. As a result, laboratory controls (H_2O and CTRL exposure chambers) were excluded from statistical analyses of survival. End-of-test (EOT) estimates of survival were then statistically compared to test the null hypothesis that there was no difference in survival of ETO between exposure chambers containing site sediments versus reference sediments. Conformation of distributions of data to approximate the normal probability function and equal variances was assessed by use of box and probability plots. If data met the assumptions then comparisons were made by use of analysis of variance (ANOVA with $\alpha = 0.05$). When assumptions for application of parametric statistical tests were violated, the non-parametric Kruskal-Wallis test was conducted ($\alpha = 0.05$). Statistical comparisons were conducted on two different permutations of the dataset. In one set of comparisons between reference and site sediments, GE and LALL sediments (references) were included in the statistical test individually or pooled. In a separate set of comparisons, an extreme value for decreased EOT survival in a UMF replicate (see section 6.4.4) was either considered or omitted during statistical comparisons between UMF sediments and reference sediments. All data analysis procedures were conducted with R version 2.9.1 (R Development Core Team 2009).

6.3.6 Length and Mass

To evaluate if growth was adversely affected at termination of the study length and mass of white sturgeon reared on site sediments were compared with those of fish reared on reference sediments. As with the survival analyses discussed in section 6.3.5, statistical procedures were conducted with the exclusion of laboratory controls. Length and mass of sturgeon surviving to

test termination were observed to vary as a function of number of fish (density) remaining at EOT. Consequently, an analysis of covariance (ANCOVA) was performed to assess the effect of site sediments on growth endpoints. An ANCOVA ($\alpha = 0.05$) was conducted with each reference site separately and with pooled references. Additional analyses were conducted with greater specificity to the secondary hypotheses, which was to determine whether fish exposed to site sediments were smaller than fish exposed to reference sediments. The approach initially taken was to conduct a nested ANOVA followed by a Dunnett's test to determine which values were significantly smaller than reference values. Results from the various statistical analyses were effectively the same, so only the ANCOVA results are described herein.

Physical and chemical characteristics of sediments were examined to explore potential causal explanations for the statistically smaller fish observed in UMF exposure chambers (see result section 6.4.5). Single variable correlation analyses were performed to identify relationships between size of fish and chemical characteristics of sediments. A multiple factor analysis (Abdi et al. 2013) was conducted in an attempt to identify discriminators, such as sediment particle size, clay and sand content of sediment, and water quality characteristics in exposure chambers, that could categorize fish by size.

6.4 Results and Discussion

Total concentrations of acid-extractable metals of concern, Cu, Cd, Pb and Zn from site sediments spanned the spectrum of concentrations observed to date within the site, and often exceeded the 90^{th} centile of previously reported data (EPA 2006 a,b), whereas concentrations

within reference sediments were statistically less, generally less than the 10th centile of site sediments (Chapter 5 Figure 5.1).

6.4.1 Toxicity of Sediments

There are several significant limitations to determining toxicity of metals in sediments and to date, there is no single, definitive method for deriving sediment quality guidelines (SQGs) or assessing risks posed by metals associated with sediments that is without limitations (Wenning et al. 2005). Empirical approaches utilize large co-occurrence databases to statistically compare chemical concentrations and biological effects under field and laboratory conditions, whereas mechanistic approaches are theoretically based and designed to predict sediment toxicity based on an understanding of the chemical and variables that influence toxicity (Wenning et al. 2005). Consensus based approaches combine previously established guidelines that used different methods of derivations but produced similar results and generate new threshold values from their central tendencies (Wenning et al. 2005). One major uncertainty in assessing potential effects of metals is bioavailability (Luthy et al. 2003). In the present study, maximum acid-extractable concentrations of metals in site sediments ranged from 712 – 3180 mg Cu/Kg DW, 1.18 – 3.56 mg Cd/kg DW, 5060 – 25600 mg Zn/kg DW, and 254 - 3410 mg Pb/kg DW. Following the consensus-based approach outlined by MacDonald et al. (2000), concentrations of metals in site sediments consistently exceeded respective threshold effect concentrations (TECs) of 31.6 mg Cu/Kg DW, 0.99 mg Cd/kg DW, 121 mg Zn/kg DW, and 35.8 mg Pb/kg DW, and PECs of 149 mg Cu/kg DW, 4.98 mg Cd/kg DW, 459 mg Zn/kg DW, and 128 mg Pb/kg DW, for the four primary metals of concern, except Cd, where most of the concentrations in site sediments ranged between the threshold and probable effect concentrations

(see Chapter 5 Figure 5.1). Based on comparisons of measured concentrations of acid-extractable metals to the TECs, exposure to site sediments from the studied region of the UCR was predicted to result in adverse effects. However, these PECs were developed to classify sediments of potential toxicity to infaunal organisms, especially benthic invertebrates and most often insects (MacDonald et al. 2000), and are not necessarily developed to evaluate potential effects on demersal fishes such as white sturgeon. Furthermore, calculations are typically based on total concentrations of acid-extractable contaminants in sediment (Burton et al. 2002) and variations in speciation and bioavailability are not normally incorporated and instead are only assumed to be accounted for through the use of large and diverse sample sets. Hence, for the present study, TEC and PEC concentrations are included to qualitatively assess the range and gradient of potential effects.

Similarly, to qualitatively assess potential effects of mixtures of metals at each sampling area, a mean PEC quotient (mPECQ) was calculated for each exposure chamber for the four primary metals of interest, and for the eight metals, arsenic (As), Cd, chromium (Cr), Cu, Pb, mercury (Hg), nickel (Ni), and Zn commonly calculated to express potential effects for mixtures of metals in sediments (MacDonald et al. 2000). The arithmetic mean mPECQ was then calculated for each sampling area from the respective replicate exposure chambers. For the four study-specific primary metals of interest mean mPECQ for site locations ranged from a minimum of 4 to a maximum of 17, whereas reference locations and controls had mPECQ values of approximately 0.04 (Figure 6.1). Considering all eight metals, mean mPECQ values for site locations ranged from a minimum of 2 to a maximum of 9, whereas reference locations and controls were approximately 0.04 (Figure 6.1). Following EPA methods of evaluation, based on

mPECQs (EPA 2000), adverse effects would be predicted for benthic-dwelling organisms following exposure to site sediments. However, caution should be taken when interpreting these predictions because they might not be fully applicable to benthic fish such as sturgeon. Also, the mPECQ method utilizes previously derived empirical sediment quality guidelines to calculate consensus-based probable effect concentrations, with their inherent limitations (Wenning et al. 2005). Issues with bioavailability, potential effects of co-occurring contaminants on individual effect concentrations, and the possibility of statistically diluting the effects of dominant toxicants when calculating mean quotients, are limitations of this method (Wenning et al. 2005). In the present study, mPECQ values for the four primary metals were greater than values calculated for all eight metals because PECQ's for As, Cr, Hg and Ni were typically lesser than for Zn, Cu, Pb and Cd and due to the calculation where sums of PECQs were divided by eight rather than four.

Mechanistic approaches to assessing risk of contaminants associated with sediments consider differences in bioavailability through equilibrium partitioning (EqP) in the interstitial water (Burton, 2002). In the present study, AVS and SEM were measured to characterize the potential toxicity of sediments contaminated with metals as part of the equilibrium sediment partitioning benchmark approach (EPA 2005). AVS and SEM are used to define excess SEM (SEMX) and carbon-normalized excess SEM (SEMX, OC; see section 6.3.3 for details of calculations and interpretations). AVS concentrations for site sediments associated with the present study were > 0.1 μmol/g, while AVS concentrations for reference and control sediments were < 0.1 μmol/g (Figure 6.2). When SEMX was calculated for site sediments from the present study values were found to be within the range of uncertainty (SEMX between 1.7 and 120 μmol/gd; Figure 6.2).

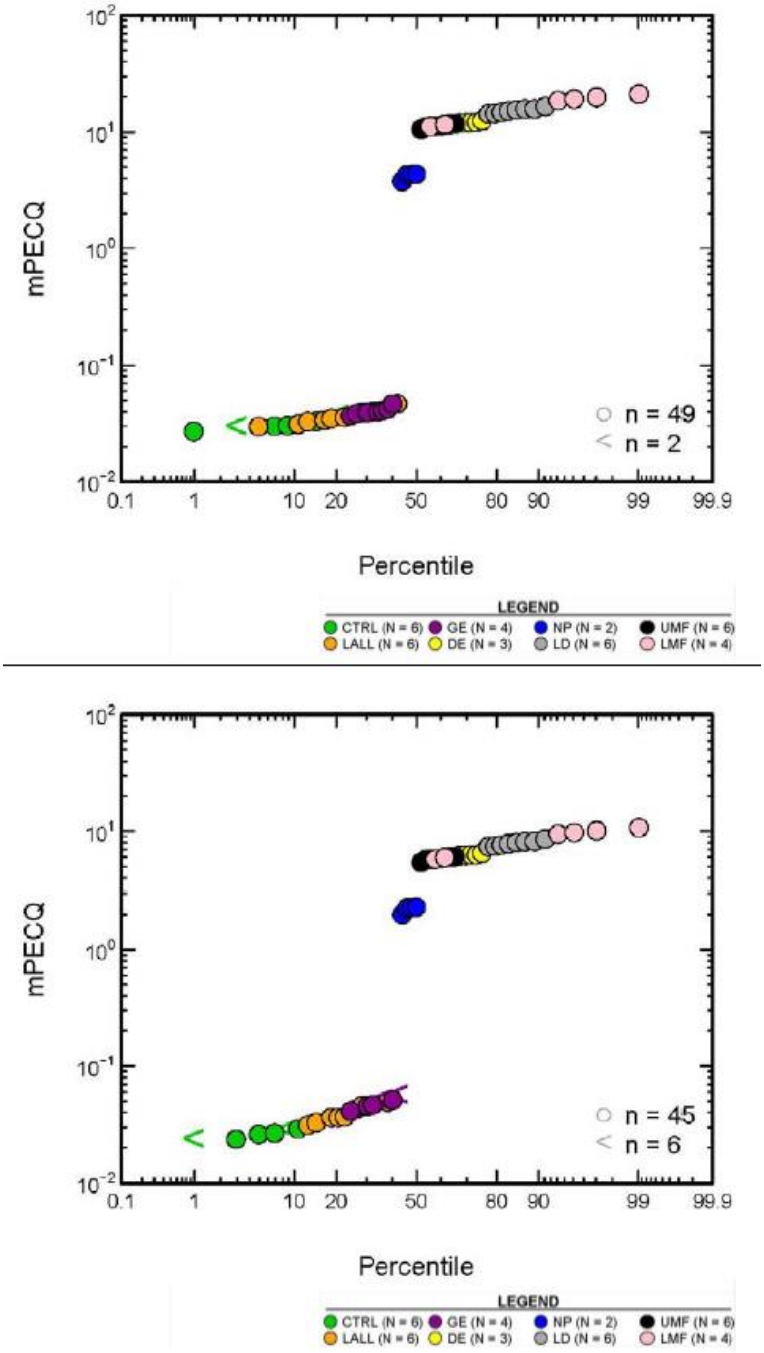

Figure 6.1. Mean probable effect concentration quotients (mPECQs) calculated for the four primary metals of interest (copper, cadmium, lead, and zinc; top panel) and for the eight metals commonly calculated to express potential effects for mixtures of metals in sediments (arsenic, cadmium, chromium, copper, lead, mercury, nickel, and zinc; bottom panel) for white sturgeon sediment toxicity tests (MacDonald et al. 2000).

As a result, these samples were further evaluated by incorporating carbon-normalization (Figure 6.3). Site sediments evaluated in the present study had relatively great carbon-normalized excess SEM (SEMX > 3000 μmol/gOC for all locations). Deadman's Eddy was found to have the greatest mean SEMX, OC value (~ 50,000 μmol/g), while Lower Marcus Flats had the least (~ 6,000 μmol/g). Based on concentrations of SEMX and OC, all site sediment areas would be expected to elicit adverse effects on sediment-dwelling organisms. As with previously discussed approaches to assess toxicity of sediments the SEM-AVS approach also has limitations (Wenning et al. 2005).

The dynamic nature of sediment influences redox status and achievement of thermodynamic equilibrium between metals and pore water, which is a major assumption of the EqP approach, and its applicability to accurate assessment of bioavailability for certain metals, such as Cu, has been questioned (Simpson et al. 1998, 2000; Simpson and Batley 2003; Wenning et al. 2005). As with all environmental assessments, the deficiencies of the methods should be evaluated and considered during risk characterization and considered in a multiple lines of evidence approach.

6.4.2 Application of Biotic Ligand Model

Application of the BLM resulted in 5,632 different BLM predictions for each primary metal of interest, Cd, Cu, Pb and Zn. Toxic units were calculated for each of the primary metals of interest in overlying water, water at the sediment water interface, and pore water at 1 and 2 cm depths. Cu was the only metal that had calculated TUs greater than 1.0, and only slightly greater in pore waters for DE substrata and LD sediments (Figure 6.4-6.7).

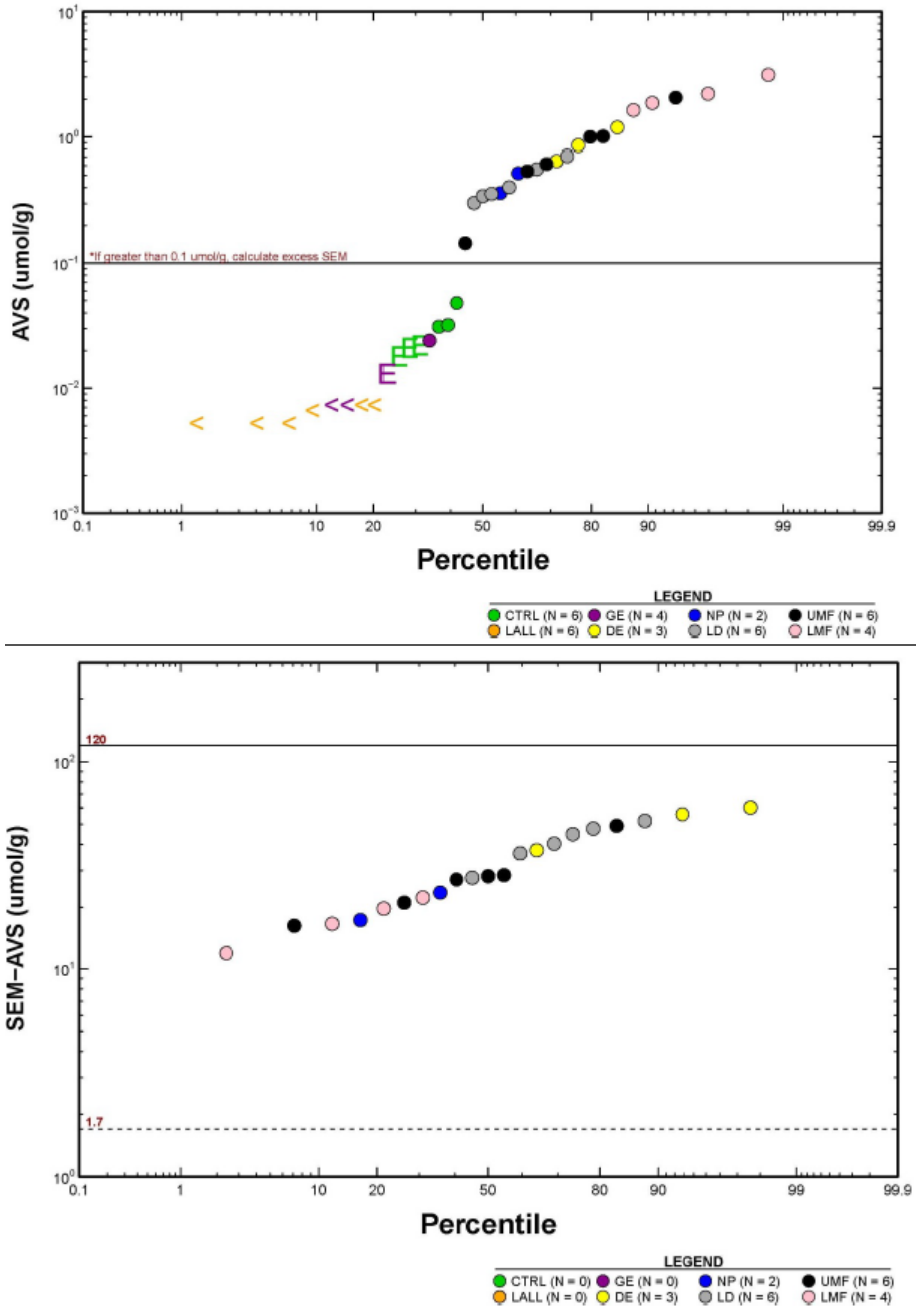

Figure 6.2. Acid volatile sulfide (AVS) levels (top panel) and excess simultaneously extracted metal (SEMX) levels (bottom panel) in sediments for white sturgeon sediment toxicity tests.

Values are calculated to characterize the potential toxicity of sediments contaminated with metals as part of the equilibrium sediment partitioning benchmark approach (USEPA 2005).

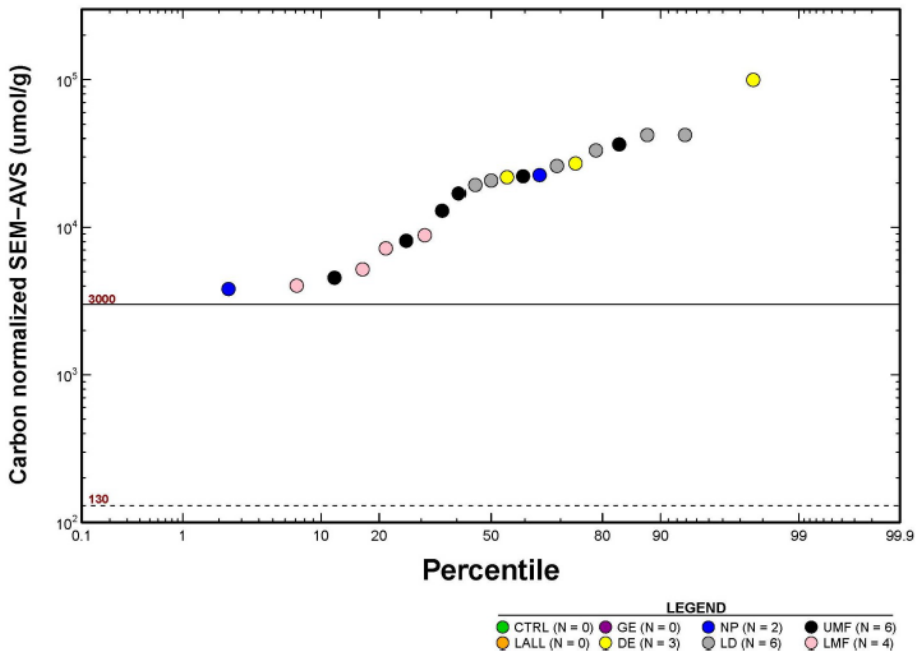

Figure 6.3. Carbon-normalized excess simultaneously extracted (SEMX) metals for the white sturgeon sediment toxicity tests.

A sediment with low carbon-normalized SEMX < 130 µmol/gOC should pose low risk of adverse biological effects due to SEMs (i.e., cadmium, copper, lead, or zinc). For sediments with high carbon-normalized SEMX >3000 µmol/gOC, adverse biological effects due to SEMs may be expected. For sediments with intermediate carbon-normalized SEMX >130 µmol/gOC and <3000 µmol/gOC there is considerable uncertainty about whether effects are expected (US EPA 2005).

TUs for Cu in pore water collected at 2.5 cm from DE exposure chambers ranged from 1.9 to 2.2. However, DE substrata were collected above the high water line (see Chapter 5), and differences in concentrations of metals between materials collected within the water versus above is not known. Exposure chambers containing LD sediments also had calculated TUs close to and in some replicates slightly in excess of 1.0 for Cu in pore waters at depths of 1 and 2.5 cm.

In the present study, TUs represent a ratio between measured concentrations of metals in a given sample and the concentration predicted by the BLM. Metal-specific BLM-predicted effect concentrations were developed using the least observed effect concentration (LC_{20}) for

survival of white sturgeon, and were based on concentrations of each metal of interest associated with a 20 % reduction in survival. Given this ratio and approach, if the calculated toxic unit is > 1.0, a 20 % decrease in survival might be expected. However, interpretation of single point-estimates of pore water TUs over the duration of the exposure is not as straightforward because for Cu, Cd, and Zn the BLM was calibrated with threshold values derived from chronic studies to estimate continuous concentrations associated with a 20 % reduction in survival. Therefore, these calculations likely represent a worst-case scenario. Nevertheless, this method provides a means of assessing risk of exposure to metals associated with UCR sediments that is more specific and applicable to early life stages of white sturgeon compared to the previous methods, such as PECs and SEMs that typically focus on effects on sediment dwelling invertebrates.

6.4.3 Survival of White Sturgeon

The target seeding density was 100 white sturgeon fry per exposure chamber; however, due to estimating the number of lost/escaping sturgeon observed at the beginning of the study, there was variability in actual seeding densities (Appendix E, Supplemental Material Table E1). During the initial period of exposure, sturgeon fry were observed to escape through small cracks in seals located near the outflow of exposure chambers. Within 48 hrs of test initiation, the seals were fixed and lost/escaping sturgeon replaced to the desired initial density of stocking. Obtaining an accurate tally of lost/escaping fish, however was difficult due to an uncertainty in the number of sturgeon fry completely flushed from exposure chambers (beyond the posterior chamber; refer to Appendix C, Supplemental Material Order 2 for a description of exposure chamber design).

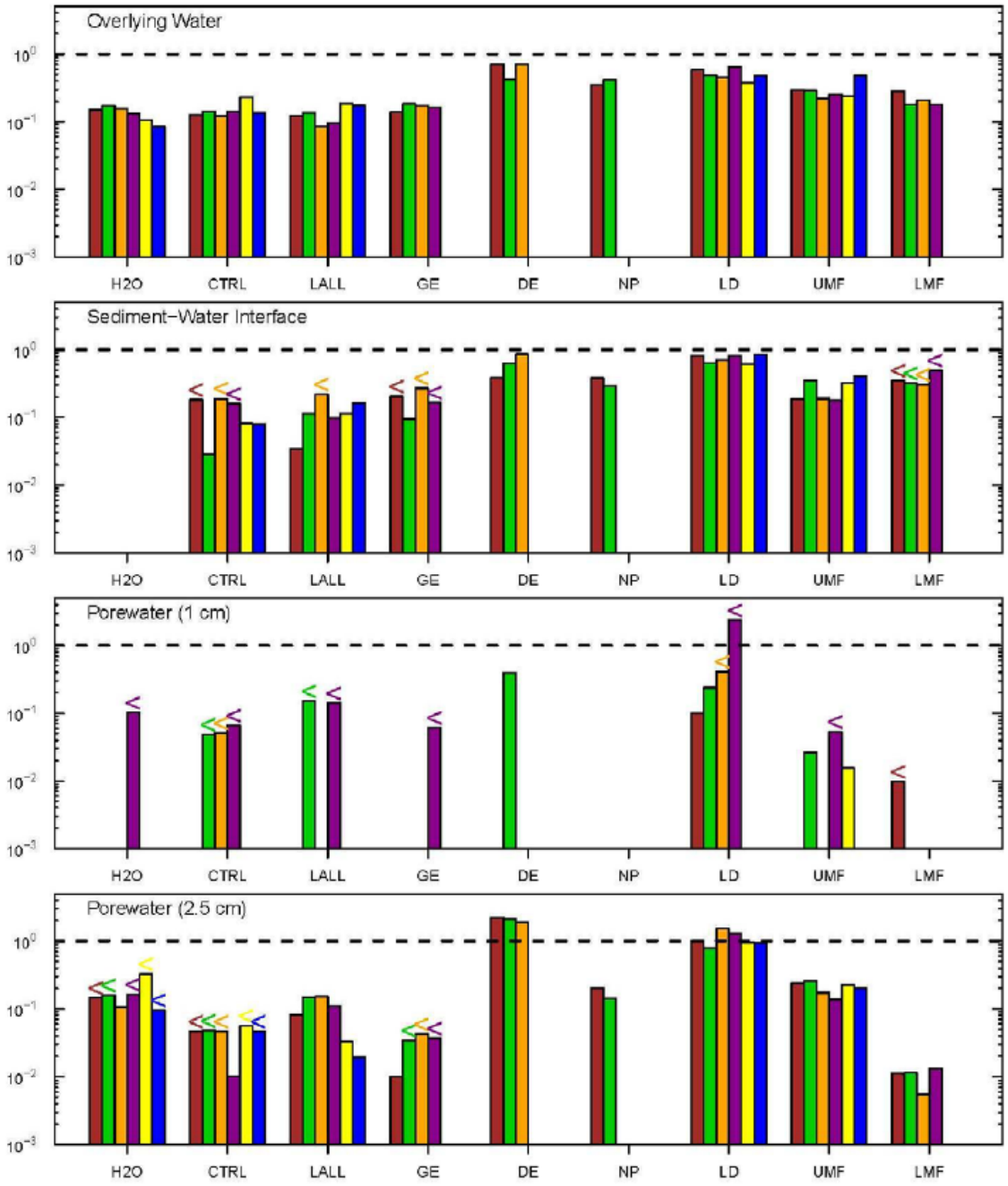

Figure 6.4. Geometric mean toxic units (TUs) for dissolved copper as a function of treatment and sample type for the white sturgeon sediment toxicity tests.

If ≥ 80% of measure concentrations were qualified (i.e., censored) maximum likelihood estimate procedures (see Vardy et al. 2014b) were used to derive geometric mean TUs. These calculations are identified with a less than symbol ("<") positioned above respective columns. Sample types (e.g., pore water) are identified in the top left-hand corner of each plot, with replicate exposure chambers illustrated with colored bars. The dashed line represents a TU of 1.

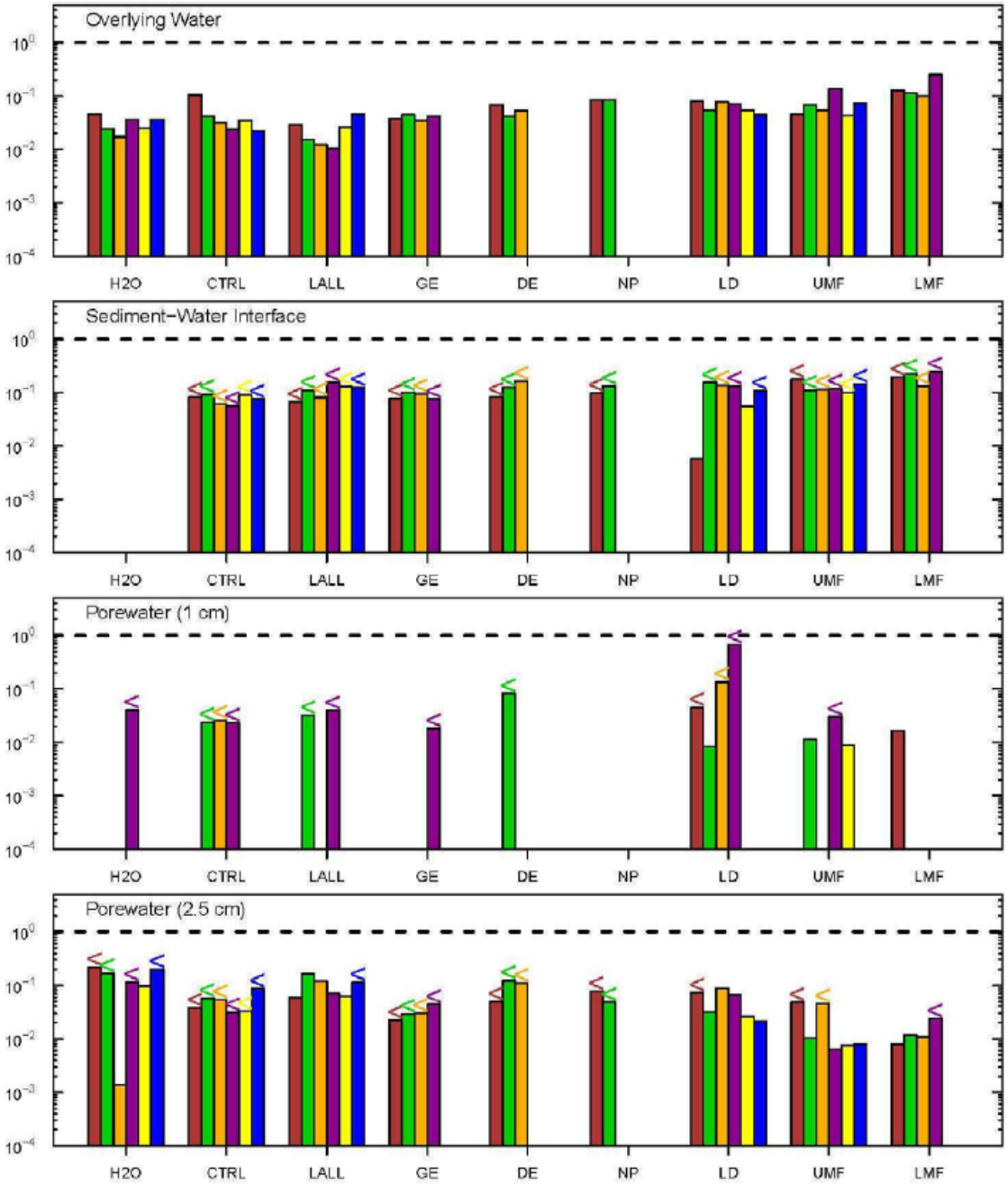

Figure 6.5. Geometric mean toxic units (TUs) for dissolved zinc as a function of treatment and sample type for the white sturgeon sediment toxicity tests.

If ≥ 80% of measure concentrations were qualified (i.e., censored) maximum likelihood estimate procedures (see Vardy et al. 2014b) were used to derive geometric mean TUs. These calculations are identified with a less than symbol ("<") positioned above respective columns. Sample types (e.g., pore water) are identified in the top left-hand corner of each plot, with replicate exposure chambers illustrated with colored bars. The dashed line represents a TU of 1.

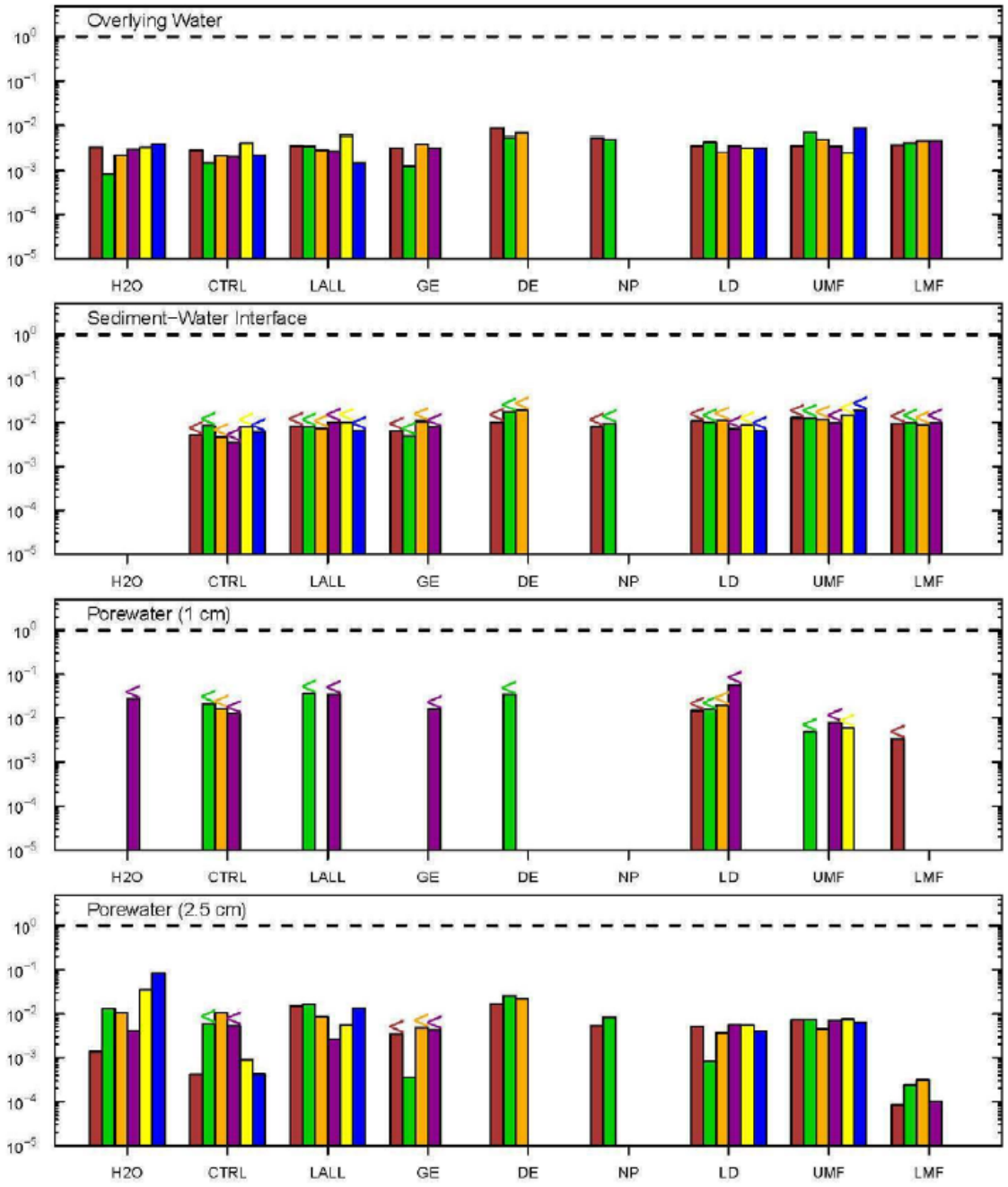

Figure 6.6. Geometric mean toxic units (TUs) for dissolved cadmium as a function of treatment and sample type for the white sturgeon sediment toxicity tests.

If ≥ 80% of measure concentrations were qualified (i.e., censored) maximum likelihood estimate procedures (see Vardy et al. 2014b) were used to derive geometric mean TUs. These calculations are identified with a less than symbol ("<") positioned above respective columns. Sample types (e.g., pore water) are identified in the top left-hand corner of each plot, with replicate exposure chambers illustrated with colored bars. The dashed line represents a TU of 1.

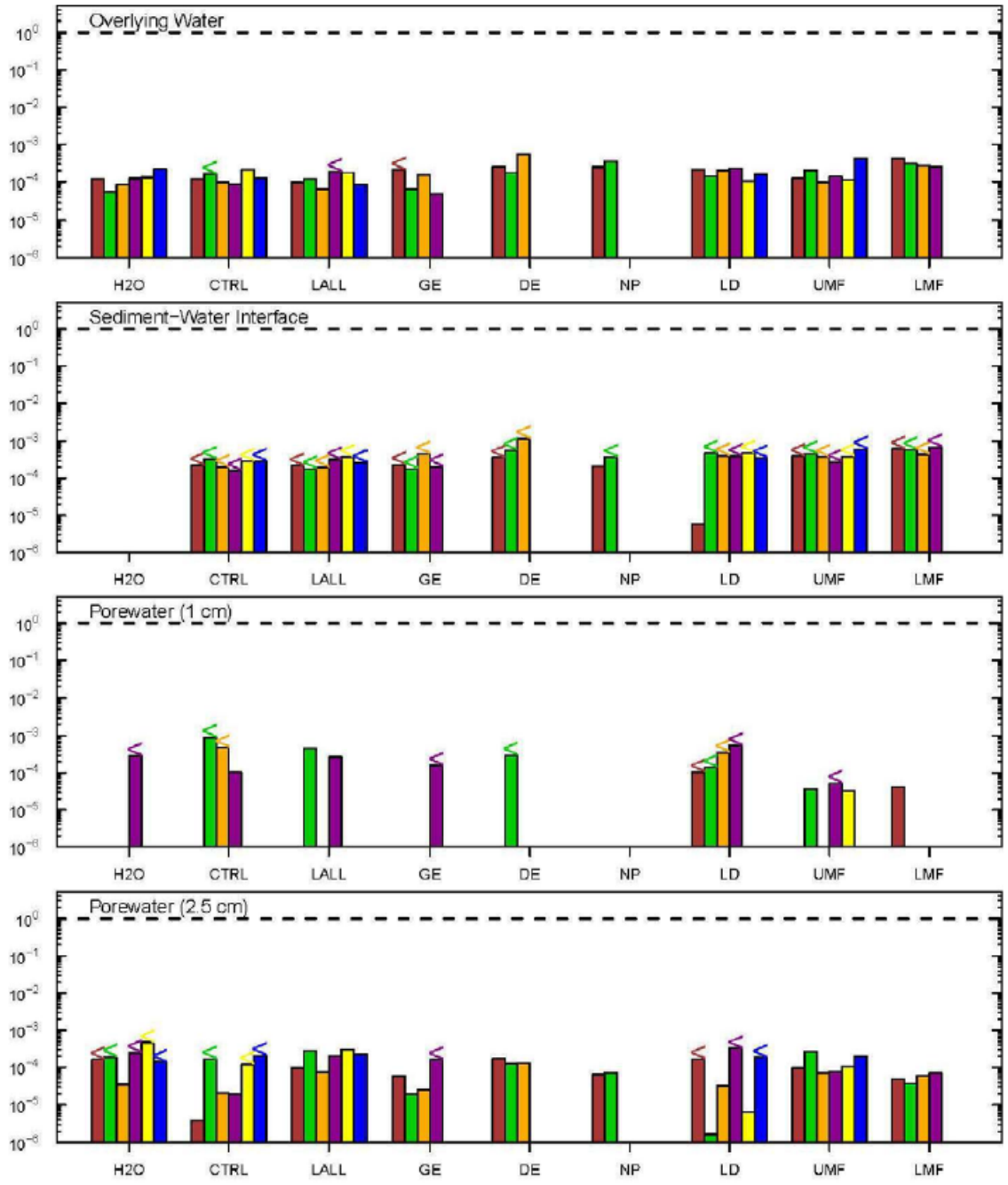

Figure 6.7. Geometric mean toxic units (TUs) for dissolved lead as a function of treatment and sample type for the white sturgeon sediment toxicity tests.

If ≥ 80% of measure concentrations were qualified (i.e., censored) maximum likelihood estimate procedures (see Vardy et al. 2014b) were used to derive geometric mean TUs. These calculations are identified with a less than symbol ("<") positioned above respective columns. Sample types (e.g., pore water) are identified in the top left-hand corner of each plot, with replicate exposure chambers illustrated with colored bars. The dashed line represents a TU of 1.

It was acknowledged that the re-seeding efforts might have resulted in differences in starting densities, but that these differences could be accounted for at the end of the study. As a result this did not adversely affect data quality or study objectives. Seeding densities were calculated by summing the number of mortalities recorded for the duration of the study, the estimated number of lost/escaping fish, and the number of surviving fish at the end of the study.

Routine cleaning operations of exposure chambers UMF-D (Day 22) and CTRL-D (Day 23) resulted in a significant loss of sturgeon fry due to breaking of seals at the outflow (see Chapter 5 section 5.4). As a result, these chambers were subsequently designated as "chemistry only" and were therefore not considered in the following analysis of biological data.

6.4.4 Survival Analyses

To minimize effects of differences in stocking densities among exposure chambers, the Kaplan-Meier method of survival analyses (see section 6.3.5) produced an EOT survival estimate that accounted for all fish introduced into exposure chambers at the onset of the study (Tompsett et al. 2014; Figure 6.8). Survival curves were consistent among replicate exposure chambers with the greatest mortalities occurring within a narrow window between 18 and 24-dph. This window coincides with the transitioning of fish to exogenous feeding and is recognized as a sensitive period of white sturgeon early life stage development (Vardy et al. 2011; Tompsett et al. 2014). Despite somewhat greater mortality during a certain window of time, overall rates of survival among exposure chambers were greater than 80%, which is in accordance with ASTM guidelines for chronic toxicity tests with early life stage fish (ASTM 2005). Exceptions were limited to two exposure chambers, a laboratory control and a site sediment. Although these two exposure

treatments are identified as having the poorest overall rates of survival (CTRL 68% survival and UMF 72%), in both instances, this was attributable to a single replicate exposure chamber (CTRL-A 17% survival and UMF-F 45% survival; see Appendix E, Supplemental Materials Figure E1). In addition to the overall survival estimates, a complete presentation of survival curves for each respective exposure chamber is provided within Appendix E, Supplemental Materials Figure E1.

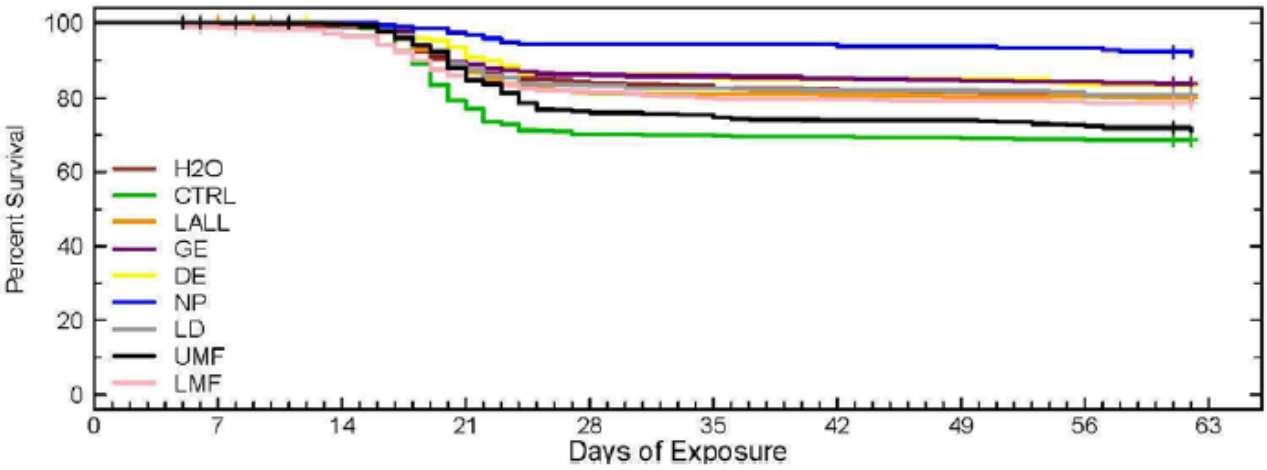

Figure 6.8. Survival analysis applied to sturgeon toxicity results from each treatment (replicates pooled) to give an overall treatment-specific survival curve for the white sturgeon sediment toxicity tests.

No statistically significant differences in survival between any of the site and reference sediment exposures were observed in any of the permutations of the statistical analysis (see section 6.3.6), with either ANOVA or Kruskal-Wallis (Appendix E, Supplemental Material Table E2). This conclusion is robust since it is supported by both the parametric as well as the less powerful nonparametric statistical tests. Therefore, in the present study, survival of white sturgeon was not adversely affected when reared on site sediments versus reference sediments.

6.4.5 Length and Mass

In addition to survival, more chronic sub-lethal effects on length and mass of white sturgeon were summarized on the basis of sediment type and exposure tank using box-and-whisker plots (Appendix E, Supplemental Material Figure E2). The overall mean mass and length of white sturgeon at EOT was approximately 0.5 g and 48 mm across all exposure chambers. Sizes in some treatments were very consistent among replicate exposure chambers, including H_2O and LALL, with greater variability in others, such as GE, LD, and UMF. Consistent with results based on survival, a laboratory control was again observed as the poorest performer. However, unlike the survival analyses, where only one replicate exposure chamber performed poorly, each replicate exposure chambers for the H_2O treatment consistently had the smallest fish with a mean length and mass of 46 mm of 0.48 g, respectively. This trend might have occurred because in laboratory exposure systems fish might perform better when housed in exposure chambers that contain natural substrata containing greater amounts of nutrients and or organics such as periphyton upon which to feed. This has been reported previously for white sturgeon exposed to industrial effluents (Tompsett et al. 2014)

Length and mass of white sturgeon surviving until termination of the test varied as a function of fish density remaining at EOT. ANCOVA was performed to assess the effect of sediment type on length and mass of white sturgeon. For the ANCOVA, it was assumed that the slope of the relationship between length or mass and the number of fish surviving at EOT was equivalent for each sediment type. This assumption was unavoidable because of the imbalance in the number of replicate exposure chambers; with only two-replicate NP exposure chambers the slope would have been strongly positive when a reasonable slope is either zero or negative. This

version of an ANCOVA used one overall slope, with an intercept for each source of sediment. Statistical comparison was based on a comparison of intercepts with a control or reference condition (Appendix E, Supplemental Material Figure E3). This relationship evaluates the assumption that size of white sturgeon in exposure chambers was density dependent. With only one exception, number of white sturgeon within exposure chambers explained all of the variation observed in size (length and mass). The intercepts from the relationship of either length or mass relative to number of fish surviving were not significantly different than the intercept for reference locations. The exception was a small but statistically significant lesser size of white sturgeon that could not be completely attributed to number of fish in the UMF treatment (ANCOVA p-values of 0.005 and 0.0039 for mass and length, respectively).

Potential causal explanations for the statistically smaller white sturgeon observed in UMF exposure chambers were explored by examining physical and chemical characteristics of sediments. In an attempt to identify relationships between size of white sturgeon and various chemical characteristics of sediments, single variable correlation analyses were performed. No strong positive correlations between length or mass of white sturgeon and concentrations of individual metals were observed. In addition, a multiple factor analysis was conducted to identify discriminators that could categorize white sturgeon fry as small, medium, or large. The multi-component discriminant analysis failed to provide descriptors that were able to predict size categories associated with the various sediments examined in this study. The difficulty in attempting to identify sediment-specific chemical or physical characteristics that can explain differences observed in size of white sturgeon might be due to the relatively small magnitude of differences in size of fish ($\leq$ 1 mm). A relationship between masses of white sturgeon at test

termination and number of fish at test termination explained 57 % of the variability. In the case of length, 53 % of the variability in size of white sturgeon was described by number of fish surviving.

6.5 Conclusions

A lines of evidence (LoE) approach applied several theoretical methods to predict empirical methods and measure effects of sediments from the UCR on survival and growth early life stages of white sturgeon. Sediments used in the present study covered a range of concentrations of targeted COPC that were representative and consistent of the range of concentrations observed in site sediments from the UCR, and captured the upper concentration range of previously reported data. Based on acid-extractable concentrations of metals, the calculated probably effect concentrations, and mean probable effect concentration quotients for metal mixtures, exposure to site sediments in the present study were predicted to have potential to result in adverse effects to benthic dwelling organisms. Similarly, based on concentrations of acid-extractable metals with principles of bioavailability, such as excess SEM, site sediments in the present study were predicted to have potential to elicit adverse biological effects due to SEMs, Cu, Cd, Pb or Zn. However, these sediment toxicity assessments, which classify sediments based on potential toxicity to infaunal organisms in sediment, such as invertebrates, and might not be directly applicable when assessing risk to a benthic fish such as white sturgeon. As a result, concentrations of metals in pore water and overlying water within exposure chambers were analyzed using the BLM to allow for more explicit consideration of bioavailability to white sturgeon, based on metal accumulated at the gill (the biotic ligand) as the basis for predicting biological effects. On the basis of calculated individual TUs, under the

laboratory conditions evaluated for this study, Cu was the only metal for which TUs were > 1.0 for a limited number of site sediments and replicate exposure chambers.

There was, however, a lack of concordance between potential for adverse effects predicted from concentrations of metals in waters and measured effects on survival and growth of white sturgeon. Survival of white sturgeon was not adversely affected by exposure to site sediments. There were statistically significant differences in growth of white sturgeon; however, these differences were largely explained by numbers of white sturgeon fry in the chamber. The smallest recorded length and mass of white sturgeon were consistently in a laboratory negative control (H_2O) group. Differences in length and mass were not associated to any of the measured chemical characteristics including concentrations of individual metals.

The sturgeon-bioassay presented in the current study avoids many of the limitations of the theoretical approaches based on both equilibrium partitioning and comparison to global sediment quality criteria. The bioassay approach considers all of the potential toxicants, including those that might not have been identified and controls for the biologically available fraction. Thus, greater weight should be given to the bioassay results than the predicted effects. In conclusion, while some of the theoretical methods indicated the potential for effects, these adverse effects were not realized in the dynamic, flow-through system applied here. Based on the result of this study, it is unlikely that exposure to UCR sediments is directly affecting survival or growth of white sturgeon.

Of the different methods used in the present study to characterize risk of exposure to metals associated with UCR sediments, BLM predictions corresponded best with survival and

growth of white sturgeon. Although use of PECs, mPECQ values, and SEMs as a means of assessing risk of exposure to metals associated with sediment to early life stage white sturgeon does not appear to be appropriate, the predictions made in the present study raise questions about potential effects of metals on the benthic invertebrate communities in the UCR and possible secondary effects on white sturgeon, such as bioaccumulation or the potential for loss of food sources. In addition, the potential effect of metal mixtures in sediments is a concern, and further investigation with UCR sediments might be merited.

CHAPTER 7

7.0 DISCUSSION and CONCLUSION

Historically, European settlers of North America had little interest in sturgeon and fishermen regarded them as no more than a nuisance fish. For Native Americans, however, sturgeon were an integral part of their culture and lifestyle, and were harvested for their meat, oil, and roe (LeBreton et al. 2004). At the turn of the nineteenth century, attitudes began to change as caviar became increasingly popular. Within a few decades, commercial fishing for white sturgeon had increased exponentially, and as a consequence, the number of reproducing adult sturgeon decreased significantly. During the period of industrialization, North American rivers began to change dramatically and anthropogenic impacts on sturgeon began to take their toll. By the second half of the twentieth century, overharvesting, alteration of habitat and pollution were threatening white sturgeon population abundances (LeBreton et al. 2004).

Over the past few decades white sturgeon research has increased. This was initially due to interests in aquaculture for caviar production, but lately efforts have focused on conservation. With many populations of white sturgeon facing possible extinction, further investigations into causes of recruitment failures are needed. Many populations of white sturgeon inhabit industrialized river systems and their life history strategies increase their potential for exposure to contaminants. Exposure to metals has been hypothesized as a potential contributing factor to poor recruitment in many of the water bodies. In general, however, little is known about the toxicity of metals to white sturgeon and their potential influence on survival of early life stages.

The objectives of the studies reported here were to develop much needed toxicity data to help characterize white sturgeon sensitivity to metals. In addition, a metal-related risk assessment was conducted on a population of white sturgeon in the Upper Columbia River (UCR). This population of sturgeon has been experiencing poor annual recruitment for over thirty years (Hildebrand et al. 2013) and was listed as endangered by the Canadian government in 2006 (COSEWIC 2013). The UCR is subject to multiple sources of pollution, including discharges from pulp and paper mills, wastewater treatment plants, and mining and smelting operations (Hildebrand et al. 2013). In particular, Teck Metals Ltd. operates a metallurgical facility in Trail, BC, Canada, that currently discharges processed effluent into the river and historically released slag, up until 1995. There are concerns that elevated concentrations of trace-elements, such as copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn), associated with the effluent and slag might have detrimental impacts on the surrounding ecosystem, including the local white sturgeon population (EPA 2006 a,b). In 2006, a remedial investigation and feasibility study (RI/FS) was initiated in the UCR, under the oversight of the US EPA (www.ucr-rifs.com), and this thesis contributed to the portion dedicated to the risk assessment of white sturgeon. This project was comprised of three major components to characterize sturgeon sensitivity to metals and risk of exposure in the UCR. Specifically, a series of dose response experiments to characterize sturgeon sensitivity to metals of concern were conducted. In addition, the effects of Teck effluent and potential contaminants within UCR water to early life stage white sturgeon were investigated. Lastly, toxicity assessments of sediments in the UCR and their effect on white sturgeon were explored.

Results from this research indicate that early life stage white sturgeon are relatively sensitive to Cu, Cd, Pb, and Zn, in comparison to other fishes. Sturgeon sensitivity to Cu is of particular interest during early life stage development when larvae are transitioning to exogenous food. White sturgeon are sensitive during this period of development (Vardy et al. 2011, 2013), and may be of increased vulnerability when exposed to metals. The threshold for effects of Cu on early life stages of white sturgeon bracketed water quality criteria for the protection of aquatic life, indicating possible issues with inadequate levels of protection (Vardy et al. 2013). There are concerns that environmentally relevant concentrations of metals, such as copper, found in water, sediment, or waters associated with sediment, such as pore water and overlying water, may approach or exceed water quality criteria and lethal concentration (LC) values for sturgeon. Previous studies (Feist et al. 2005; Kruse and Scarnecchia 2002a; UCWSRI 2002) have hypothesized that the presence of contaminants in rivers could be contributing to failures in recruitment of sturgeon.

This project provided a unique opportunity to investigate multiple routes of potential exposure of metals to early life stage white sturgeon within a riverine environment. In the UCR the potential exists for exposure to metals in the water column, in sediments, and in different water matrices associated with sediments. The initial phase of the risk assessment portion of this project characterized the effects of contaminants in water downstream of the metal smelter in Trail, BC, Canada. Results indicated that Teck effluent is not likely to adversely affect survival or growth of early life stage white sturgeon, at environmentally relevant concentrations. Teck effluent is significantly diluted to less than 1% of its original concentration once it is discharged into the river system and toxicity would not be expected at the major spawning site where early

life stages of sturgeon are likely to be present and where the riverside experiments from the present project were conducted. Comparison of concentrations of metals in the Columbia River with concentrations that have been shown to be chronically toxic to early life stage white sturgeon (Vardy et al. 2011) indicated that current concentrations of waterborne metals downstream of the metal smelter at Trail, BC, are unlikely to be a causative factor for the recruitment failures of sturgeon in the river. However, other potential routes of exposure to metals in the UCR were identified and investigated.

Contaminants associated with sediments in the UCR and their impact on survival of sturgeon is of particular concern. Previous studies have analyzed sediments from the UCR and have found elevated concentrations of Cu, Pb, Cd, and Zn, relative to reference sites, downstream of the metallurgical facility (Besser et al. 2008; Bortleson et al. 2001; EPA 2006 a,b). Early life stages of sturgeon are closely associated with the benthos and previous studies have hypothesized that sturgeon may be at risk of exposure to elevated concentrations of metals associated with sediments in the UCR (Paulson and Cox 2007). To address these concerns, the present study characterized UCR sediment samples that were collected downstream of the smelter in locations previously determined to be contaminated with metals and known to be white sturgeon spawning- and/or nursing-grounds (EPA 2006 a,b). Chemical analyses of whole sediment samples revealed significantly elevated concentrations of metals of primary interest, including Cu, Cd, Pb, and Zn. However, these metals appeared to be largely sequestered in the sediment and not bioavailable to sturgeon. Results of exposure experiments indicated that survival of white sturgeon was not adversely affected following exposure to UCR sediments. Although it appears unlikely that exposure to UCR sediments is directly related to sturgeon

mortality, secondary effects may be an issue. Based on concentrations of metal in whole sediment samples from the UCR, sediment toxicity assessments such as probable effect concentrations (PECs) and excess simultaneously extracted metals (SEMX) predict adverse effects to sediment dwelling organisms; a potential food source for early life stage sturgeon. Bioaccumulation of metals or the potential for loss of food sources for early life stage sturgeon are issues that merit further investigation.

Predictive and preventative tools to aid in risk assessment and remediation efforts are of great value. However, the present project identified deficiencies in current methods and practices in North America that could lead to imprecise estimates of risk to fishes such as sturgeon. When possible, regulatory agencies need to adopt water quality guidelines and criteria for metals that are site specific. Differences in water quality variables have been demonstrated to modify metal toxicity to fishes and therefore should be considered when developing water quality objectives. In addition, sensitive species and sensitive life stages should be considered in order to ensure adequate protection for species of concern. White sturgeons, for example, are more sensitive to metals such as Cu after the initial yolksac life stage when transitioning to exogenous food (chapter 2), and current water quality criteria does not consider this and consequently are under protective in the state of Washington. As new research and technology is developed and proven effective, outdated methods and guidelines need to be revisited. Metal speciation models such as the Biotic Ligand Model (BLM) are commonly used and for some metals have been demonstrated as effective means of predicting toxicity to various aquatic organisms based on ambient water quality parameters. In the US and Canada, however, only the EPA utilizes the BLM when calculating water quality criteria, and only for Cu.

The ability to translate laboratory results to predictive effects in the field has long been debated and is an ongoing challenge. In situ experiments like the whole water river experiments presented in chapter 4 are often conducted to capture site specific conditions that may alter toxicity in a given environment. In order to accurately derive water quality standards, water effect ratios (WERs) are often calculated by conducting side by side acute toxicity experiments in laboratory water and field water (Stephan et al. 1994). The present project conducted a modified version of the WER experiment whereby white sturgeon were raised and cultured separately in laboratory water and field water prior to toxicity tests (chapter 3). Results indicated that sturgeon cultured in river water likely acclimatized to greater concentrations of calcium and subsequently were more tolerant to exposure to metals during toxicity tests. This could have significant implications when deriving site specific water quality standards as fish in the field are likely to have adapted to the characteristics of their environment. This presents yet another challenge when trying to translate laboratory tests to field conditions and merits further investigation.

To date, there is no definitive method for deriving sediment quality guidelines or assessing risk to benthic fishes that are in close association to contaminated sediment. The present project employed a variety of approaches, including both mechanistic and consensus-based approaches, as well as a sediment bioassay to assess risk to white sturgeon exposed to Columbia River sediment. Typically, during risk assessments of sediment, it is common practice to compare total concentrations of acid-extractable metals in sediment to PECs, and to calculate excess simultaneously extracted metals (SEMX; Fairbrother et al. 2007). Results from the present project, however, indicated that while these approaches might be suitable methods for

assessing risk to benthic dwelling organism, such as invertebrates, they are not necessarily appropriate for assessing risk to benthic dwelling fish such as sturgeon (chapter 5 and 6). Alternatively, it was determined that use of the BLM resulted in a more accurate assessment of risk to sturgeon. In the present project, concentration-response relationships between dissolved metal and observed mortality, normalized for bioavailability by considering accumulation on the biotic ligand (i.e. gill), were developed. From the concentration-response on the biotic ligand, the median lethal accumulations, or LA_{50}s, were estimated. Site-specific water quality parameters from porewater or overlying water can then be used in combination with the white sturgeon BLM parameter files and the calculated LA_{50} to predict thresholds for effects or calculate toxic units for field sediments. Although not perfect, the BLM appears to be a superior method overall for calculating risk of contaminated sediment to benthic fish, and when feasible, risk assessors should consider its use when performing site assessments. In addition, site specific water quality variables can be used with the sturgeon-modified BLM parameter files to normalize concentration-response relationships or predict thresholds for effects, such as LC_{50}s, in different field scenarios. This would greatly aid in risk assessment and regulation not only in the UCR, but in any water body where white sturgeon are at risk of exposure to metals.

Research from this project provided valuable information to help assess potential causes for poor recruitment of white sturgeon. There are many hypotheses as to why population numbers of white sturgeon have been decreasing in the UCR over the last few decades, but as of yet, no definitive cause for poor recruitment has been identified. Without identification of the specific factors responsible for the failures, it has not been possible to remediate the cause to allow for natural recruitment. As a consequence, white sturgeon have been listed as endangered

in parts of Canada and the USA. Collaborative efforts between both countries are essential in hopes of restoring healthy, self-sustaining populations. Groups such as the White Sturgeon Recovery Initiative play crucial roles in maintaining and aiding current white sturgeon populations, and research efforts have increased in the past few years in attempts to better understand their decline. As more research is conducted, possible causes for recruitment failure can start to be eliminated. Based on results from this risk assessment, metals in the UCR do not appear to be contributing directly to decreased survival of early life stage sturgeon. It was determined, however, that sturgeon are sensitive to metals, and potential issues with under-protective water quality criteria were identified that need to be addressed.

The effect of habitat alteration is a major issue that merits further investigation. The river channel and watershed of the Columbia River have been modified from their historical conditions. There are 14 major hydroelectric dams on the river and 46 in the watershed. Damming can cause sedimentation and result in the loss of appropriate spawning substratum or areas for fry to develop and find refuge from predation. Predation by introduced species such as walleye (*Sander vitreus*) may also be a contributor to poor recruitment. In addition, impoundment of the river by dams can alter water quality variables and primary productivity, affecting food sources for early life stages of sturgeon. It is not likely that a single causative factor, but rather a combination of issues, are contributing to poor recruitment of white sturgeon. Future studies that simultaneously address multiple factors, such as altered flow-regimes, habitat, water temperatures, and turbidity are necessary. Such studies would be difficult and intensive to perform, but would ultimately be needed to understand why populations of these ancient fish have declined over so much of their range.

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APPENDIX A

CHAPTER 2 SUPPLEMENTAL MATERIALS

Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper

Table A1. Summary of Mean ± Standard Deviation of Water Quality Parameters.

Parameter	Analysis	WS 8dph	WS 15dph	WS 40dph	WS 45dph	WS100dph	RT 8dph	RT 15dph	RT 40dph	RT 45dph	FM 8dph
Temperature (˚C)[b]	UofS	16.2 (±0.41)	15.1 (±0.02)	15.0 (±0.04)	15.1 (±0.03)	14.6 (±0.29)	13.2 (±0.34)	11.5 (±0.07)	13.3 (±0.38)	13.1 (±0.13)	21.5 (±0.08)
pH (s.u.)	UofS	7.6 (±0.18)	7.7 (±0.06)	7.5 (±0.06)	7.2 (±0.07)	7.5 (±0.18)	7.9 (0.05)	7.4 (±0.09)	7.7 (±0.07)	7.7 (±0.01)	7.9 (±0.03)
DO (%)	UofS	79 (± 10.3)	83 (±1.3)	94 (±2.7)	85 (±3.6)	89 (±1.9)	95 (±5.2)	77 (±1.3)	103 (±2.6)	84 (±3.4)	94 (±3.0)
Conductivity (µS/cm)	UofS	183 (±5.4)	186 (±5.1)	176 (±44)	164 (±2.3)	213 (±2.4)	187 (±1.7)	164 (±6.1)	165 (±2.2)	187 (±13)	185 (±2.7)
Ammonia Nitrogen (mg/L)	UofS	< 0.02*	<0.02*	0.03 (±0.02)	<0.02*	0.11 (±0.14)	0.03 (±0.02)	0.04 (±0.01)[a]	< 0.02*	0.04 (±0.04)[b]	< 0.02*
Nitrate (mg/L)	UofS	<0.25*	<0.25*	0.29 (±0.08)	<0.25*	0.33 (±0.14)	<0.25*	<0.25*	<0.25*	<0.25*	<0.25*
Nitrite (mg/L)	UofS	<0.02*	<0.02*	<0.02*	<0.02*	< 0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*
Hardness (mg/L)	CAS	60 (±2.3)	64 (±2.5)	54 (±2.5)	60 (±1.2)	63 (±1.5)	56 (±2.1)	53 (±3.4)	45 (±2.0)	62 (±2.9)	61 (±0.4)
Alkalinity (mg/L)	CAS	40 (±8.7)	38 (±2.8)	31 (±1..2)	37 (±0.7)	31 (±0.9)	33 (±0.7)	33 (±2.7)	31 (±0.9)	35 (±3.5)	33 (±0.7)
DOC (mg/L)	CAS	2.2 (±0.50)	1.6 (±0.14)	2.2 (0.23)	2.2 (±0.11)	2.2 (±0.20)	1.9 (±0.21)	2.0 (±0.09)	2.2 (0.20)	1.5 (±0.08)	1.9 (±0.21)
Ca^{2+} (µg/L)	CAS	9.6 (±0.52)	12 (±0.45)	9.0 (±0.23)	11 (±0.38)	9.0 (±0.13)	9.2 (±0.19)	9.8 (±0.73)	9.0 (±0.13)	11 (±0.93)	9.2 (±0.19)
Mg^{2+} (µg/L)	CAS	7.8 (±0.17)	8.1 (±0.35)	7.7 (±0.18	8.1 (±0.10)	7.8 (±0.10)	7.7 (±0.11)	7.0 (±0.36)	7.8 (±0.10)	8.1 (±0.25)	7.7 (±0.11)
Na^{+} (µg/L)	CAS	13 (±0.31)	14 (±0.59)	12.7 (±0.22)	14 (±0.25)	12.8 (±0.16)	12.8 (±0.20)	11 (±0.46)	12.8 (0.16)	13 (±0.50)	12.8 (±0.20)
K^{+} (µg/L)	CAS	1.5 (±0.05)	1.6 (±0.07)	1.4 (±0.02)	1.7 (±0.02)	1.4 (±0.02)	1.5 (±0.05)	1.3 (±0.07)	1.4 (±0.02)	1.6 (±0.07)	1.5 (0.05)
SO_4^{-} (µg/L)	CAS	46 (±3.4)	45(±3.2)	41 (±1.7)	49 (±1.2)	42 (±0.6)	43 (±0.7)	41 (±3.1)	42 (±0.6)	46 (±3.1)	43 (±0.7)
Cl^{-} (µg/L)	CAS	5.6 (±0.13)	8.7(±2.4)	5.5 (±0.08)	5.8 (±0.14)	5.5 (0.06)	5.5 (±0.10)	7.4 (±0.80)	5.5 (±0.06)	7.8 (±1.6)	5.5 (0.10)

WS = White Sturgeon; RT = Rainbow Trout; FM = fathead minnow; dph = Days Post Hatch; DO = Dissolved Oxygen; DOC = Dissolved Organic Carbon

Analysis – Refers to laboratory by which analyses were conducted

* Limit of Detection, all or majority of values below this value

[a] 11% of all values were below the LOD of 0.02 mg/L

[b] 54% of all values were below the LOD of 0.02 mg/L

Table A2. Analytical methods and associated method detection limits for samples collected from the acute toxicity experiments with white sturgeon and rainbow trout.

Parameter	Method	Laboratory	Unit	LOD
Copper	EPA 6020	CAS	µg/L	0.02[a]
Temperature	VWR Symphony 14002-860	UofS	˚C	0
pH	VWR Symphony 14002-860	UofS	s.u.	0
DO	VWR Symphony 11388-374	UofS	mg/L	2
Conductivity	VWR Symphony 11388-372	UofS	µS/cm	1
Ammonia-Nitrogen	LaMotte Kit 3304	UofS	mg/L	0.02
Nitrate	LaMotte Kit 3319	UofS	mg/L	0.25
Nitrite	LaMotte Kit 7674	UofS	mg/L	0.02
Hardness	SM2340C	CAS	mg/L	0.8
Alkalinity	SM2320B	CAS	mg/L	1.0
DOC	SM5310C	CAS	mg/L	0.07
Ca^{2+}	EPA6010B	CAS	µg/L	6
Mg^{2+}	EPA6010B	CAS	µg/L	0.3-2.0
Na^{+}	EPA6010B	CAS	µg/L	20
K^{+}	EPA6010B	CAS	µg/L	40-50
SO_4^{-}	EPA300.0	CAS	µg/L	0.01-0.1
Cl^{-}	EPA300.0	CAS	µg/L	0.06-0.1

CAS = Parameter measured by Columbia Analytical Services; DO = Dissolved oxygen; DOC = Dissolved organic carbon; LOD = Limit of detection; UofS = Parameter measured by University of Saskatchewan

[a] With exception of two data points in the 15 dph white sturgeon experiment at 96h for which the LOD was 0.33

Table A3. Water quality parameter measurements in field blanks (nanopure water) and analytical method blanks (CAS) used in the acute toxicity experiments with white sturgeon and rainbow trout.

Parameter	Method	Unit	Field Blank[a]	Method Blank
Copper	EPA 6020	µg/L	0.04-0.2	0.02-0.07
Ammonia-Nitrogen	LaMotte Kit 3304	mg/L	<0.02[b]	[c]
Nitrate	LaMotte Kit 3319	mg/L	<0.25[b]	[c]
Nitrite	LaMotte Kit 7674	mg/L	<0.02[b]	[c]
Hardness	SM2340C	mg/L	<0.4-1.0	<0.04
Alkalinity	SM2320B	mg/L	<1	<1-6
DOC	SM5310C	mg/L	0.64-3.73	0.07-0.15
Ca^{2+}	EPA6010B	µg/L	<6.7-66	<6-13
Mg^{2+}	EPA6010B	µg/L	<0.3-20	<0.3-2
Na^{+}	EPA6010B	µg/L	<20-46	<20
K^{+}	EPA6010B	µg/L	<44	<44-78
SO_4^{-}	EPA300.0	mg/L	<0.02-0.74	<0.02-0.04
Cl^{-}	EPA300.0	µg/L	0.06-0.35	0.03

DO = Dissolved oxygen; DOC = Dissolved organic carbon; LOD = Limit of detection

[a]Values represent range of blanks from all experiments with exception of the 15dph rainbow trout experiment, for which no blanks were collected

[b]Blanks for ammonia-nitrogen, nitrate, and nitrite test kits (LaMotte) were only analyzed in the 15dph white sturgeon experiment

[c] Method and Field Blanks are identical because these measurements were made directly in the lab using test kits (LaMotte)

APPENDIX B

CHAPTER 3 SUPPLEMENTAL MATERIALS

Acute toxicity of copper, lead, cadmium, and zinc to early life stages of white sturgeon (*Acipenser transmontanus*) in laboratory and Columbia River water

Table B1. Summary of mean ± standard deviation of water quality parameters.

Metal		Copper				Lead				Cadmium		Zinc	
Life stage		8 dph		40 dph		8 dph		40 dph		8 dph		8 dph	
Exposure Water	Analysis	Lab	CR	Lab	CR	Lab	CR	Lab	CR	Lab	CR	Lab	CR
Temperature (°C)	U of S	16.2 (±0.41)	16.0 (±0.43)	15.0 (±0.04)	16.3 (±0.25)	15.8 (±0.61)	16.7 (±0.45)	15.0 (±0.15)	16.3 (±0.21)	16.8 (± 0.1)	15.5 (± 1.2)	16.8 (± 0.09)	15.5 (± 1.2)
pH (s.u.)	U of S	7.6 (±0.18)	7.7 (±0.23)	7.5 (±0.06)	7.8 (±0.24)	7.6 (±0.20)	7.7 (±0.28)	7.4 (±0.23)	7.9 (±0.26)	7.4 (± 0.07)	7.8 (± 0.35)	7.3 (± 0.05)	7.8 (± 0.35)
DO (%)	U of S	79 (± 10.3)	101 (±17)	94 (±2.7)	103 (±6.6)	83 (±8.2)	98 (±15)	94 (±2.6)	99 (±8.0)	77 (± 6.1)	82 (± 5.4)	76 (± 1.9)	82 (± 5.4)
Conductivity (µS/cm)	U of S	183 (±5.4)	127 (±7.6)	176 (±44)	130 (±2.5)	181 (±30)	126 (±8.6)	188 (±39)	130 (±2.3)	224 (± 5.8)	140 (± 0.35)	225 (± 5.1)	140 (± 0.35)
Ammonia Nitrogen (ppm)	U of S	< 0.02*	<0.025*	0.03 (±0.02)	<0.025*	<0.025*	0.031 (±0.048)*	<0.025*	<0.025*	<0.025*	<0.025*	<0.025*	<0.025*
Nitrate (ppm)	U of S	<0.25*	<0.25*	0.29 (±0.08)	<0.25*	<0.25*	<0.25*	<0.25*	<0.25*	<0.25*	<0.25*	<0.25*	<0.25*
Nitrite (ppm)	U of S	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.01*	<0.01*	<0.01*	<0.01*
Alkalinity (ppm)	CAS	40 (±8.7)	52 (±0.58)	31 (±1. 2)	52 (±3.0)	33 (±2.2)	53 (±0.61)	30 (±2.1)	54 (±1.2)	50 (± 0.3)	62	50 (± 0.3)	62
DOC (mg/L)	CAS	2.1 (±0.25)[#]	1.8 (±0.16)[#]	2.1 (0.25)[#]	1.9 (±0.10)[#]	2.1 (±0.30)[#]	1.8 (±0.09)[#]	1.9 (± 0.14)[#]	1.8 (±0.14)[#]	2.6 (± 0.31)	2.3 (± 0.14)	2.8 (± 0.44)	2.3 (± 0.14)
Ca^{2+} (µg/L)	CAS	9.2 (±0.19)	16.7 (± 0.29)	8.9 (±0.19)	18.0 (±0.2)	9.6 (± 0.3)	16.9 (± 0.3)	9.7 (± 1.7)	17.7 (± 0.2)	15.4 (±0.2)	20.0 (± 0)[δ]	15.4 (± 0.5)	20.0 (± 0)[δ]
Mg^{2+} (µg/L)	CAS	7.8 (±0.17)	3.8 (±0.07)	7.8 (±0.14)	4.0 (±0.03)	8.1 (±0.15)	3.9 (±0.08)	8.3 (± 1.4)	3.9 (± 0.04)	9.1 (± 0.1)	5.0 (± 0)[δ]	9.1 (± 0.3)	5.0 (± 0)[δ]
Na^{+} (µg/L)	CAS	13 (±0.20)	1.3 (± 0.04)	12.8 (±0.18)	1.4 (±0.03)	13.1 (±0.17)	1.2 (±0.02)	13.6 (± 2.4)	1.4 (± 0.03)	10.5 (± 0)	2.5 (± 0)[δ]	10.5 (± 0)	2.5 (± 0)[δ]
K^{+} (µg/L)	CAS	1.5 (±0.05)	0.6 (± 0.03)	1.5 (±0.03)	0.6 (±0.01)	1.5 (± 0.07)	0.6 (±0.02)	1.6 (± 0.31)	0.6 (± 0.01)	1.0 (± 0)	0.7 (± 0)[δ]	1.0 (± 0)	0.7 (± 0)[δ]
SO_4^{-} (µg/L)	CAS	44 (±0.71)	10 (±0.2	41 (±1.7)	9.9 (± 0.1)	46 (± 1.4)	10.2 (± 0.1)	41 (± 1.0)	9.8 (± 0.1)	50.0 (± 0)	10.0 (± 0)	50 (± 0)	10.0 (± 0)
Cl^{-} (µg/L)	CAS	5.5 (±0.09)	0.7 (± 0.1)	5.5 (±0.1)	0.9 (± 0.1)	5.5 (± 0.1)	0.7 (± 0.1)	5.6 (± 0.2)	0.7 (± 0.2)	6.3 (± 0)	1.0 (± 0)	6.3 (± 0)	1.0 (± 0)

Analysis – refers to laboratory by which analyses were conducted

* Limit of detection, all or majority of values below this value

[#] Due to contamination, TOC was used instead of DOC

[δ] Parameter was estimated from a related exposure and study (Tompsett et al. unpublished data)

Table B2. Analytical methods, method detection limits, and water quality parameter measurements in field blanks (nanopure water) and analytical method blanks (CAS) used in the acute toxicity experiments with white sturgeon.

Analytical Methods and Associated Method Detection Limits				
Parameter	Method	Unit	LOD	
University of Saskatchewan Analysis				
Cadmium	EPA ILM05.2D	µg/L	0.0031	
Copper	EPA ILM05.2D	µg/L	0.068	
Zinc	EPA ILM05.2D	µg/L	0.18	
Temperature	VWR Symphony 14002-860	°C	0	
pH	VWR Symphony 14002-860	s.u.	0	
DO	VWR Symphony 11388-374	mg/L	2	
Conductivity	VWR Symphony 11388-372	µS/cm	1	
Ammonia-Nitrogen	LaMotte Kit 3304	mg/L	0.05	
Nitrate	LaMotte Kit 3319	mg/L	0.25	
Nitrite	LaMotte Kit 7674	mg/L	0.02	
Hardness	LaMotte Kit 4824-DR-LT	mg/L	20	
Alkalinity	LaMotte Kit 4419-DR	mg/L	20	
Sulfate	LaMotte Kit 7778	mg/L	20	
DOC	EPA 415.3	mg/L	0.01	
Columbia Analytical Services Analysis				
Copper	EPA 6020	µg/L	0.02	
Lead	EPA 6020	µg/L	0.005	
Hardness	SM 2340C	mg/L	0.8	
Alkalinity	SM 2320B	mg/L	1	
DOC	SM 5310C	mg/L	0.07	
Ca^{2+}	EPA6010B	µg/L	6	
Mg^{2}	EPA6010B	µg/L	0.3-2.0	
Na^{+}	EPA6010B	µg/L	20	
K^{+}	EPA6010B	µg/L	40-50	
SO_4^{-}	EPA300.0	µg/L	0.01-0.1	
Cl^{-}	EPA300.0	µg/L	0.01-0.1	
Water Quality Measurements in Blanks				
Sample Type	Parameter	Method	Unit	Value/Range
Reverse Osmosis Water	DOC	EPA 415.3	mg/L	0.22
City of Saskatoon Tap Water	DOC	EPA 415.3	mg/L	2.98
Columbia River Water	Cadmium	EPA 415.3	µg/L	0.026-0.09
Columbia River Water	Copper	EPA 415.3	µg/L	0.72-0.88
Columbia River Water	Zinc	EPA 415.3	µg/L	8.8-22
Method Blank	Cadmium	EPA ILM05.2D	µg/L	≤ 0.037
Method Blank	Cadmium	EPA ILM05.2D	µg/L	≤ 2.159
Method Blank	Copper	EPA ILM05.2D	µg/L	< LOD
Method Blank	Copper	EPA ILM05.2D	µg/L	≤ 0.36
Method Blank	Zinc	EPA ILM05.2D	µg/L	≤ 0.74
Method Blank	Zinc	EPA ILM05.2D	µg/L	< LOD
Field Blank	DOC	SM 5310C	mg/L	0.32 - 1.5
Field Blank	TOC	SM 5310C	mg/L	0.14 - 0.41
Field Blank	Copper	SM 5310C	mg/L	<0.02 - 0.2
Field Blank	Lead	SM 5310C	mg/L	0.026 - 0.323
Method Blank	DOC	SM 5310C	mg/L	n/a
Method Blank	TOC	SM 5310C	mg/L	<0.07 - 0.37
Method Blank	Copper	SM 5310C	mg/L	<0.02 - 0.03
Method Blank	Lead	SM 5310C	mg/L	<0.005 - 0.01
Columbia River Water	Copper	EPA 6020	µg/L	0.71 - 1.1
Columbia River Water	Copper	EPA 6020	µg/L	0.63 - 0.79
Columbia River Water	Lead	EPA 6020	µg/L	0.09 - 0.19
Columbia River Water	Lead	EPA 6020	µg/L	0.15 - 0.40
Columbia River Water	Copper	EPA 6020	µg/L	0.37 - 0.39
Columbia River Water	Copper	EPA 6020	µg/L	1.0 - 1.2
Columbia River Water	Lead	EPA 6020	µg/L	0.044 - 0.12
Columbia River Water	Lead	EPA 6020	µg/L	0.084 - 0.53
Field Blank	Ca^{2+}	EPA6010B	µg/L	<6.7-66
Method Blank	Ca^{2+}	EPA6010B	µg/L	<6-13
Field Blank	Mg^{2+}	EPA6010B	µg/L	<0.3-20
Method Blank	Mg^{2+}	EPA6010B	µg/L	<0.3-2
Field Blank	Na^{+}	EPA6010B	µg/L	<20-46
Method Blank	Na^{+}	EPA6010B	µg/L	<20
Field Blank	K^{+}	EPA6010B	µg/L	<44
Method Blank	K^{+}	EPA6010B	µg/L	<44-78
Field Blank	SO_4^{-}	EPA300.0	mg/L	<0.02-0.74
Method Blank	SO_4^{-}	EPA300.0	mg/L	<0.02-0.04
Field Blank	Cl^{-}	EPA300.0	µg/L	0.06-0.35
Method Blank	Cl^{-}	EPA300.0	µg/L	0.03

APPENDIX C

CHAPTER 4 SUPPLEMENTAL MATERIALS

Effects of Columbia River water on early life-stages of white sturgeon (*Acipenser transmontanus*)

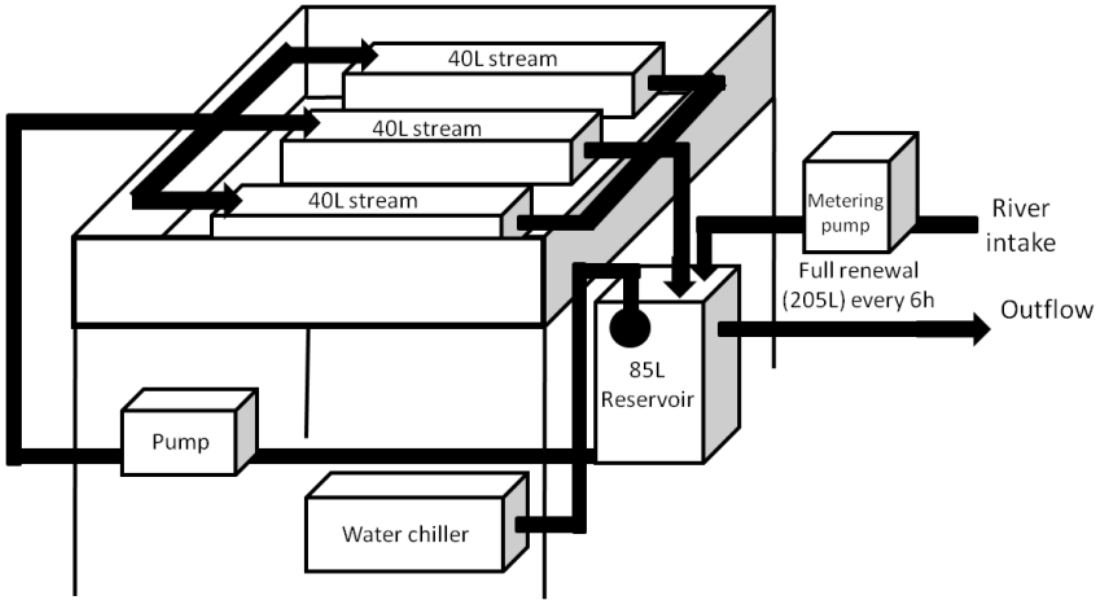


Figure C1. Schematic of flow-through system for Columbia River whole water exposures.

Water entered the system through the river intake, where it was metered into an 85L reservoir at a rate sufficient to replace the water in the entire system every 6 h. Water in the reservoir was constantly chilled to ~15°C. Reservoir water was pumped into a manifold that split the water into 3-40L streams, or chambers. Water was delivered to the streams via nozzles with the flow directed to maximize mixing. Water exited the chambers via tubing and was deposited back into the 85L reservoir, where it was recirculated through the system. As river water was metered into the 85L reservoir, recirculated water was gradually replaced.

Culture of early life stages of white sturgeon

In both 2008 and 2009, mortality of early life-stages of white sturgeon was dependent on initial stocking density. As such, the relationship between the number of fish that died during the experiment and stocking density was explored. Mortality from control treatments from a previous study (Vardy et al., 2011) were included in the analysis, and it was determined that the relationship between initial stocking density and mortality was consistent between the two studies. A least squares linear regression was performed with initial stocking density as the independent variable and total number of fish that died during the experiment as the dependent variable. The experimental unit was the treatment chamber, which was assumed to be a true replicate for this analysis. A significant ($p<0.001$) correlation that explained most of the variation was observed between the two parameters ($r^2=0.98$; Figure C2).

Based on the relationship between stocking density and mortality, recommendations for culture of white sturgeon for studies using similar design parameters were developed. Flow rate did not affect the relationship between initial stocking density and mortality, so the culturing recommendations were based on density or surface area required by each fish when it is undergoing the transition to exogenous feeding, which was the stage during which most sturgeon died. Calculations were based upon the average number of fish to survive to exposure termination, as well as the average mass of a fish when transitioning to exogenous feeding in 2009, because these figures led to the most conservative estimates of recommended density and surface area. The chamber volume and surface area were the same for all experiments. The recommended initial stocking density is presented based on mass per unit volume (Equation C1) and recommended surface area is presented as area per fish (Equation C2).

Recommended stocking density = 86 fish*0.049 g/fish /40 L/chamber = 0.11 g/L (Equation C1)

Recommended surface area = 5000 cm^2 / 86 fish = 58 cm^2/fish (Equation C2)

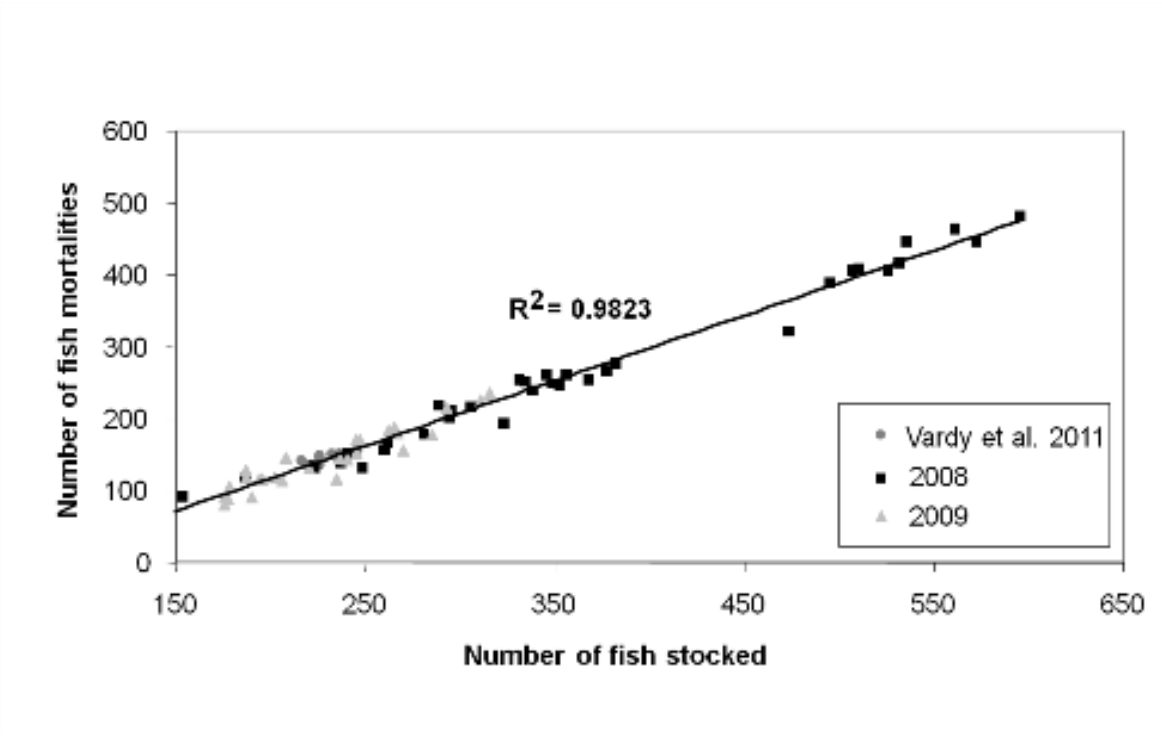


Figure C2. Regression of number of fish stocked vs. number of fish mortalities in Columbia River whole water exposures.

For the 2008 and 2009 field studies and for control fish from a concurrent laboratory study (Vardy et al., 2011), there was a significant linear relationship between the number of fish stocked into each chamber at experiment initiation and the number of fish deaths over the course of the experiment (r^2=0.98; $p<0.001$).

APPENDIX D

CHAPTER 5 SUPPLEMENTAL MATERIALS

Assessment of Columbia River sediment toxicity to white sturgeon: concentrations of metals in sediment, pore water, and overlying water

Method Development Work

Prior to the assessment of toxicity of metals associated with sediment in the Columbia River to early life stages of white sturgeon, methods were developed and evaluated of a flow-through fluvial simulation system at the University of Saskatchewan's (UofS) Aquatic Toxicology Research Facility (ATRF) that was specifically designed for use in studies of toxicity of sediments to early life stages of white sturgeon. The primary goals of the method development were to evaluate and confirm the performance of the flow-through fluvial exposure system and to establish suitable control sediments. Specifically, an experimental exposure system was needed to allow for adjustment of flow velocity, water replacement time, recirculation frequency, sediment thickness, and pore water sampling volume and depth. The method development work was aimed at establishing uniform flow conditions to minimize "dead spaces" at the inflow and outflow of the exposure chambers, to establish which hydrological operating conditions (i.e. flow) would result in the smallest gradient between pore water and overlying water, and what was the time to steady-state. In addition, the effects of different volumes and distributions of substratum on hydrological conditions in the chambers were examined, as well as the influence of sediment depth, depth of pore water sampling within the sediment layer, and sampled volume on exchange between overlying water and pore water. Optimum techniques for homogenization of sediment, sampling of pore water and system cleaning techniques were also investigated.

Results from methods development work and associated final study design are presented herein and summarized (Table D1).

Table D1. Summary of method development work for toxicity assessment of metals associated with sediment in the Columbia River to early life stages of white sturgeon.

Order	Parameter	Goal	Test Conditions	Measurement	Recommendation
1	Sediment Mixing	Confirm the effectiveness and reliability of sediment homogenization procedures.	Samples collected from the gravel bar at Deadman's Eddy were mixed and homogenized in a specially designed 'concrete mixer'.	6 sediment samples collected following mixing and analyzed for concentrations of Cu, Cd, Zn, and Pb. Sediment considered homogenized if concentrations of metals within ± 20 percent the maximum calculated difference.	The cement mixer an effective method of mixing sediment. Samples tumbled for 3 hours, stopping every half an hour to rotate the drum and scrap any sediment from the sides before repeating the process.
2	Experimental exposure systems	Establish an appropriate exposure system to test potential toxicity of sediments to early life stage white sturgeon	Flow-through fluvial simulation system for chronic exposures of early life stage sturgeon to sediment; ability to sample matrices associated with sediment	Effectively allow for adjustment of flow velocity, water replacement time, recirculation frequency, sediment thickness, and pore water sampling volume and depth.	A flow-through HDPE re-circulation system was established
3	Flow condition	Establish parameters and operational conditions that enable the maintenance of homogenous flow conditions in the test system	Initial flow rate of 19 L/min, with incremental changes of +/- 2 L/min to achieve desired end state	Video record of fluorescein dye movement	Initial flow rate of 20 L/min to accommodate low flow requirement for yolksac larvae, and then increase flow rates to 25 L/min around the time when larvae initiate exogenous feeding
4	Gravel volume and distributions	Establish optimum density of gravel to create pseudo-hyporheic zone	Gravel: 0, 3, 7, 10 and 13 stones per 100 cm^2	Conductivity measurements	4 stones per 100 cm^2
5	Pore water sampling	Establish pore water sampling methods	Airstone suction device in different depths of sediment using variable strength and duration of suction (via manual use of syringe). Initial volume to be collected 30 mL, with incremental changes of ± 5 mL to obtain sufficient sample volume	Only pore water is collected with no overlying water in the sample • Dye concentrationmeasurements	12 ports with a volume of 8-10 ml each; no ports within the first and last 4 inches of the fluvial chamber
6	Sediment depth	Establish optimum depth of sediment for early life stage sturgeon and to maximize pore water collection	Initial depth at 2 inches, with trials of 3 and 4 inches	Pore water sampling at 0.5 and 1 inch and overlying water sampling within the 1 cm of water overlying the sediment • Dye concentration measurements	Two (2) inches of sediment, with airstones positioned on top of 0.5 inches and below 1.5 inches of sediment

7	Gradients between pore and overlying water	Establish operational conditions that minimize gradients in water quality parameters between pore- and overlying water	Each flow/sediment depth combination that is tested	Time-resolved measurements of: • Dye concentration • Conductivity • DOC • pH	Flow-rates between 17 and 25L are appropriate as they do not affect gradients. Pore water sampling depth between 1 and 1.5 inches recommended due to observed gradients. Short time-dependency of gradients between overlying water and pore water indicates that a reduced equilibration time of 4 to 7 days prior to introduction of fish is sufficient
8	Time to steady-state	Establish operational conditions that minimize time to steady-state	Characterize time to steady state between pore- and overlying water after establishing optimal flow and gravel conditions	Time-resolved measurements of: • Alkalinity • Ammonia • Conductivity • DO • DOC • Hardness • pH	48 hours is sufficient for all parameters, with the exception of DOC which may not reach steady state
9	Cleaning methods	Establish most efficient method for cleaning	Introduce food 3X daily and scrape tanks at days 2, 3, 4 and 5.	Measure turbidity of samples using light scattering methods	Modified pipette, with spatula used to remove biofilm, if necessary
10	Laboratory control sediment	Define clean sediment with characteristics similar to UCR sediments	Research lab controls used in other bioassays. Create sediment from clean silica sand and/or granite with grain size 0.5 to 2 mm and preference to dark color	Measure grain size and color	Control sediment: Hagen Geosystem Black Fine Gravel (ART #12648), all analytes below screening ecological values (SEVs). Acceptable for use. Reference sediments from Genelle and Lower Arrow Lake, gravelly sand, all analytes below SEVs. Acceptable for use

Order 1: Sediment mixing and homogenization

All sediments, including site sediments, reference sediments, and artificial control sediments, needed to be mixed thoroughly to ensure homogeneity of the material, prior to layering them into the fluvial exposure chambers. The following sections summarize the work conducted during methods development to arrive at the definitive mixing method and duration.

Objective: Confirm the effectiveness and reliability of sediment homogenization procedures

Experimental Design

Samples collected from the gravel bar at Deadman's Eddy were mixed and homogenized in a specially designed 'concrete mixer'. The large rotating drum of the mixer contained a plastic liner that had been tested to confirm lack of leaching of metals into a water rinsate. Composited sediment was tumbled for a period long enough (e.g., hours) to create a visual appearance of complete mixing. Two sediment samples each were taken from the top, middle, and bottom layers of the drum and analyzed for Cu, Cd, Pd, and Zn to verify the visual determination of homogenized sediment. If analyses had greater than ± 20 percent maximum calculated difference in concentration, then the sample was tumbled for another period and the analysis repeated. Analysis of metal concentrations was conducted by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) at the University of Saskatchewan, Saskatoon, SK, Canada, following methods outlined by Creed et al. (1994).

Decision Criteria

Sediments were determined to be completely homogenized when all six samples collected were within ± 20 percent the maximum calculated difference. Photographs of homogenized sediment were taken to document the visual appearance of samples at homogeneity.

Results

Rinsate:

The cement mixer was scrubbed with 5% HCl, followed by Liqui-Nox (Sigma-Aldrich, Oakville, ON, Canada), and thoroughly rinsed with reverse osmosis (RO) water. 50 L of

nanopure water was then tumbled in the mixer for a period of 3 hours. The average of two rinsate samples (ppb), ± standard deviation, for Cu, Zn, Cd and Pb were 0.68 ± 0.33, 0.92 ± 0.2, 0.1 ± 0.02 and 0.03 ± 0.01, respectively.

Homogenization:

Two sets of six samples were analyzed for homogeneity, for a total of 12 data points for each metal of concern. The decision criteria for sediment homogenization stated that the 6 samples, two from the top, middle and bottom of the mixer, were to be analyzed for Cu, Zn, Cd, and Pb and that concentrations ≤ 20% of the mean of the six samples would be considered acceptable. Cu, Zn and Cd results all were well below the 20% criterion, whereas Pb results were not. When comparing all 12 samples, Pb exceeds 20% 6 times (50%, 48%, 26%, 33%, 33% and 21%).

Conclusions

Results for Cu, Cd and Zn indicated that the sediment achieved homogeneity after three hours mixing time. The cement mixer appeared to be an effective method of mixing sediment and it was used for mixing sediments for the definitive study. Samples were tumbled for 3 hours, stopping every half an hour to rotate the drum and scrap any sediment from the sides before repeating the process.

The probable cause for the greater variation in concentrations of Pb among subsamples than was observed for Cu, Zn or Cd, is that Pb was likely present in part as larger solid particles as opposed to associated with fines as surface oxides and bound to organic ligands. Since

concentrations of Cu, Zn and Cd all indicated that the bulk sediment was homogenized, the greater variation in concentrations of Pb was likely due to the size of the sub-subsample collected for quantification. A sub-subsample of 0.1 g dry weight (dw) was taken for digestion. If a larger sub-subsample had been collected and digested, and the digestate diluted prior to analysis, it is likely that the variation among sub samples would be less. Since Pb is generally occluded in the solid matrix of particles it is less likely to contribute directly to toxicity in pore water. This conclusion is supported by the results reported by Besser et al. (2008) for location L7 in the Columbia River, which is in the vicinity of Deadman's Eddy. In that study of the toxic potential of metals in sediments from the UCR, the concentration of Pb in bulk sediment from L7 was 590 μg Pb/g dw while the concentration in the pore water was 7.1 μg Pb/L. At other locations such as L6, which is downstream from L7, the concentration in the bulk sediment was 200 μg Pb/g, dw but the concentration in the pore water was 250 μg Pb/L. A fractionation of the sediment at L7 showed that more than 50% of the Pb was in the residual unextractable fraction and an additional 40% was present as a sulfide (or organic), but most likely an insoluble sulfide material. These results taken together indicate that Pb in the vicinity of Deadman's Eddy is more likely to be bound in the matrix of the sediments and less likely to contribute to lead concentrations in pore water. The volume of bulk sediment added to the experimental systems was such that the variation observed in very small sub-subsamples will not be observed among exposure systems. It can be concluded based on the results for Cu, Cd and Zn that the sediments were sufficiently homogenized to be used in further experimentation. Following mixing, sediments were placed into 5 gallon high density polyethylene (HDPE) buckets, overlaid with water, and stored at 4°C under a nitrogen atmosphere until placement into the fluvial system chambers.

Order 2: Experimental exposure systems

Objective: Establish an appropriate exposure system to test potential toxicity of sediments to early life stage white sturgeon

Experimental Design

Test systems comparable to those used in the present study had been previously employed at the UofS ATRF in chronic experiments with white sturgeon exposed to aqueous metals (Vardy et al. 2011). However, the test systems had not been used or specifically tested for the purpose of conducting flow-through sediment toxicity tests. Specifically, parameters associated with the design, such as fluvial chamber dimensions and layout, location of sampling devices, delivery, re-circulation, and chilling of water, and other operational conditions were established and tested to inform the definitive study design.

Results

Different designs and various prototypes of exposure chambers were tested. For the definitive study, artificial flow-through exposure chambers were constructed from high density polyethylene (HDPE) and screens were fabricated from plexi-glass with fiberglass mesh (Figure D1). A head tank contained the reverse osmosis and dechlorinated lab water mixture supplied to the experimental exposure systems (Figure D2). The mixture was delivered from the head tank to the 85-L exposure system reservoir via a metering pump. The mixture delivery rate from the reservoir to the exposure chambers was regulated by a delivery manifold attached to a re-circulating march pump. The mixture flowed from the delivery manifold at the exposure

chamber inflow, through the exposure chamber, and exited through outflow drain holes, which in turn were connected back to the 85-L reservoir. There was an overflow drain at the back of each reservoir that discarded wastewater and a baffle within each reservoir that prevented short-circuiting of the inflow to the overflow drain. The water was cooled to the desired temperature by placing a chiller unit inside the 85-L reservoir. The delivery manifold at the inflow of the exposure chamber and four drains with ball valves at the outflow of the exposure chamber allowed for adjustments to flow regime. Ports were built into the side of the exposure chambers to connect airstones for pore water sampling.

Conclusions

An experimental exposure system was established that allowed for adjustment of flow velocity, water replacement time, recirculation frequency, sediment thickness, and pore water sampling volume and depth.

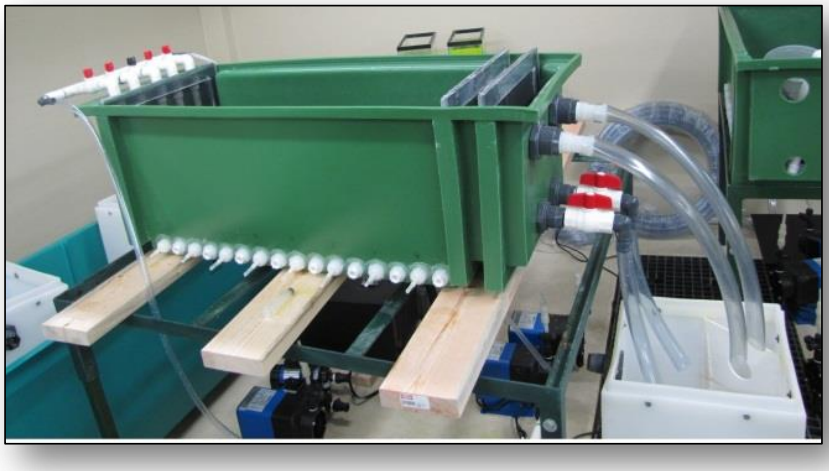


Figure D1. Flow-through exposure chamber for testing of toxicity of sediments to early life stage white sturgeon.

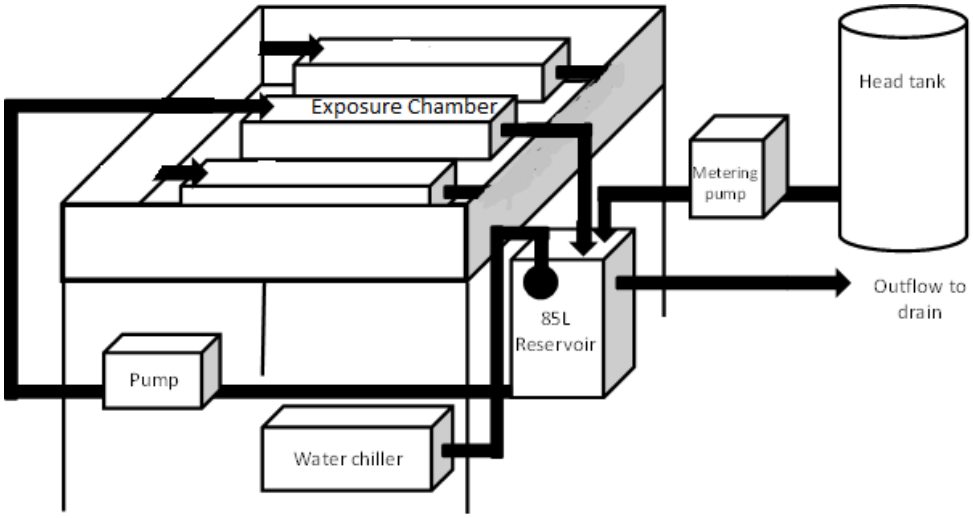


Figure D2. Schematic of flow-through system layout showing 3 exposure chambers in Upper Columbia River sediment toxicity tests.

Order 3: Determination of optimum flow rate in fluvial exposure chamber

Objective: Evaluate and establish homogenous water flow conditions to ensure uniform distribution of inflow and flow within the posterior chamber, and to minimize "dead spaces" at inflow and outflow of exposure chamber.

Experimental Design

A fluorescent dye (Fluorescein) was used to measure water flow; such dyes are cost effective and easily and accurately measured with a fluorometer and observed with an ultraviolet (UV) lamp. Flow-rates ranging from 5 to 25 L/min were tested in duplicate. After the dye was introduced into the test chamber (t = 0), it was made visible by UV lighting, and dispersal of dye and associated water flows were recorded by means of a digital video camera across the entire chamber. Additionally, at flow rates that appeared acceptable as gauged against the goal of this

experiment (i.e., ≥17 L/min), water samples were taken at t = 10 sec (intake), t = 20 sec (middle) and t = 30 sec (outflow) at 3 locations equally distributed over the cross-section of the chamber. This was repeated for cross-sections close to the inflow, centre, and outflow of the test chamber, resulting in a total of 3 x 9 = 27 water samples. The first and last sampled cross-sections were located at the inflow and outflow screens of the sediment exposure chamber, respectively, to identify potential dead spaces (see Figure D3). Samples obtained during the second experiment (flow-rates ≥17 L/min) were analyzed for dye concentrations using a microtiter plate fluorescent reader (Polastar Optima, BMG Labtech, Offenburg, Germany), and dye concentrations were mapped throughout the chamber. Sampling was conducted using 10 mL pipettes modified such that samples could be taken at different depths throughout the chambers. Dye concentration experiments during which water samples for fluorometer quantification were collected were run either in two (17 and 25 L/min) or three (20 L/min) replicates.

Data Presentation

Descriptive statistics (mean ± SD) of relative dye intensities. Fluorescence measurements are expressed relative to the maximum fluorescence intensity (FLU) measured during each experiment (FLU/maximum FLU). Assessment and evaluation of water flow conditions within the exposure chamber was also based on visual observations and records (e.g., video).

Results

The dye experiment revealed a significant impact of flow rate on the uniformity of flow throughout the test chamber (Table D2). Flow rates less than 17 L/min caused uneven flows that were biased towards one side of the test chamber (Figure D4 A&B). Flow rates equal to or

greater than 17 L/min resulted in even flows across the chamber both horizontally and vertically (Figure D4 C&D). When measuring dye concentrations using the fluorometer, there was still some remaining variation at a flow rate of 17 L/min (Figure D5A). At flow rates greater 17 L/min there were only minor differences in fluorescent dye concentrations across sampled cross-sections regardless of the position in the chamber (Figure D5 B&C)

Table D2. Assessment of flow properties based on video-dye experiment for methods development of Upper Columbia River sediment toxicity tests.

Flow Rate	**Comment**
5 L/min	Uneven flow, too much variability
8 L/min	Uneven flow, too much variability
13 L/min	Difficulties in obtaining acceptable flow conditions
17 L/min	Acceptable flow conditions
20 L/min	Acceptable flow conditions
25 L/min	Acceptable flow conditions

Conclusions

Stable and homogenous flows were achieved at flow rates greater than 17 L/min. It was therefore recommended to initiate the study with a flow rate of 20 L/min to accommodate low flow requirement for yolksac larvae, and then increase flow rates to 25 L/min when larvae initiated exogenous feeding, and were large enough to easily withstand the increased flows. The proposed flow-rates resulted in ground velocities that were less than those occurring in the UCR, and did not impact sediments layered into the chambers (e.g. causing re-suspension).

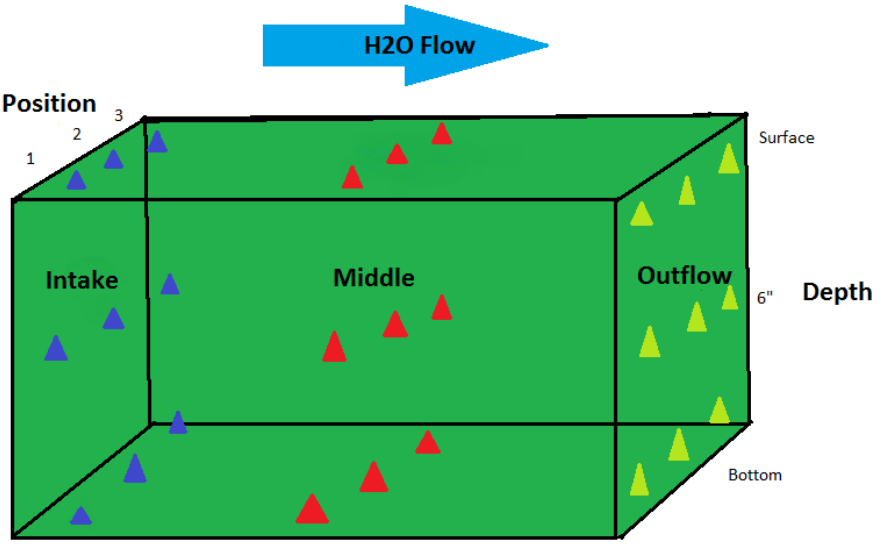


Figure D3. Illustration of sampling point distribution for collecting water samples throughout an exposure chamber during the flow condition experiment for methods development of Upper Columbia River sediment toxicity tests.

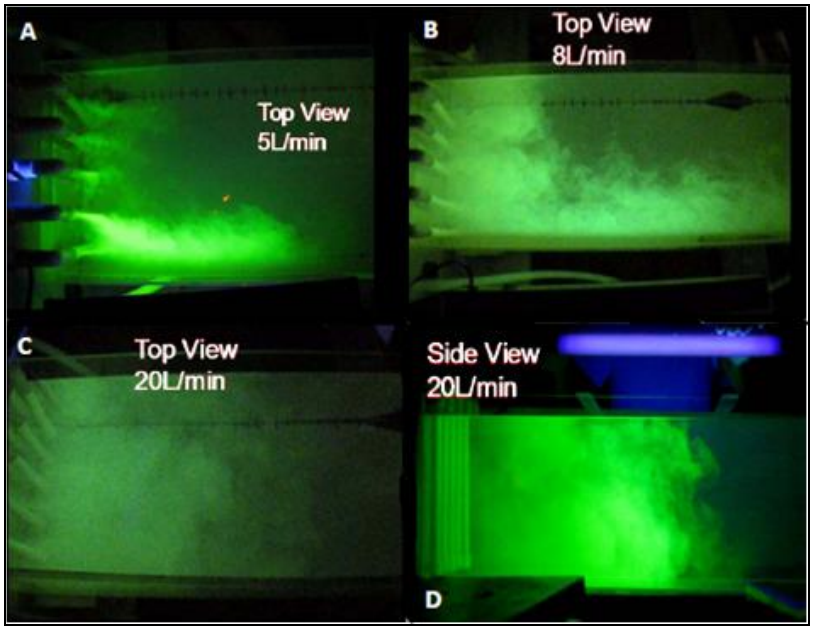


Figure D4. Photographs of dye distribution in test chamber at low (< 17 L/min; A&B) and high (≥ 17 L/min; C&D) flow rates for methods development of Upper Columbia River sediment toxicity tests.

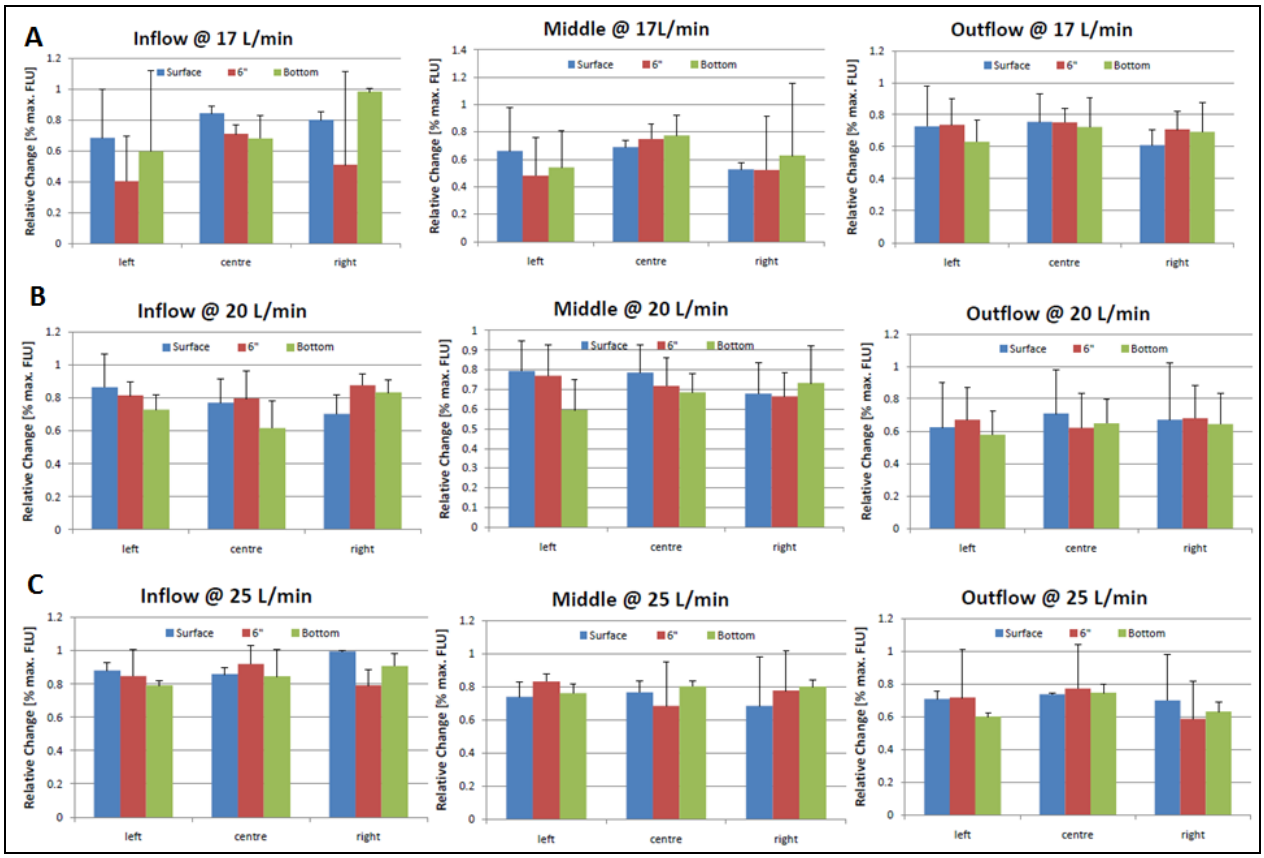


Figure D5. Mean ± 1 standard deviation relative dye concentrations at sampling points in cross sections sampled at the inflow, middle, and outflow of the test chamber at flow rates of 17 (A), 20 (B) and 25 (C) L/min for methods development of Upper Columbia River sediment toxicity tests.

Cross sectional position in exposure system (viewed from inflow end of test chamber): 1=left; 2=centre; 3=right. Values are expressed relative to the maximum dye concentration measured in the same experiment (maximum dye concentration = 1).

Order 4: Determination of optimum stone volume and distribution in fluvial test chambers

Objective: Indentify the effect of different volumes and distribution of stones on hydrological conditions in fluvial chambers.

Experimental Design

1. Stone volume selection

Stones (Geosubstrate # 12422, Hagen) (10 to 20 mm diameter) were placed in exposure chambers without sediment to visually assess optimal spatial densities. Spatial densities of stones tested included: 0, 3, 5 and 7 stones per 100 cm^2. Tests with densities greater than 7 stones per 100 cm^2 were not included because of crowding issues.

2. Water quality evaluation

Conductivity measurements were taken at 0, 24 and 48 hrs in the 1 cm water layer overlying the stones to assess variation in water quality. Stone densities of 0, 3, 5 and 7 stones per 100 cm^2 were tested in exposure chambers without sediment while stone densities of 0, 4 and 7 stones per 100 cm^2 were tested in exposure chambers with sediment. Sediments were layered at a thickness of 2 and 3 inches into the exposure portion of the test chambers and stones were placed on top. Sediment depths greater than 3 inches were not included based on the evaluation of optimum sediment depths; refer to summary Table D1.

3. Flow condition evaluation

Flow condition was evaluated visually by use of fluorescent dye to determine if the stones altered the flow of water.

Decision Criteria

Conductivity measurements to evaluate variation in water quality: target value of <30% variation among samples taken. Visual assessment of flow conditions using fluorescent dye.

Results

1. Stone volume selection

Visual assessment of stone densities of 3 and 7 stones per 100 cm^2 appeared too sparse and too crowded, respectively, whereas stone densities of 4 and 5 per 100 cm^2 appeared appropriate leaving sufficient room to enable observations of sediments while providing appropriate refuge for white sturgeon early life stages (Figure D6).

2. Water quality evaluation

Conductivity analysis revealed less than 15% variation among samples taken from all stone densities at 0, 24 and 48 hrs in exposure systems with and without sediment (Figure D7 and 8). Variation in conductivity was less than 10% in exposure systems with sediment and stone densities of 4 and 7 stones per 100 cm^2 (Figure D8).

3. Flow condition evaluation

Visual assessments of flow condition revealed no impact on water flow with the incorporation of stones into the exposure chamber or with increased stone densities in chambers with or without sediment.

Conclusions

Addition of stones to the fluvial exposure chambers had no impact on flow conditions and little to no impact on water quality. In our experience early life stages of white sturgeon appear less stressed when provided a refuge under experimental conditions. A density of 4 stones per 100 cm^2 was recommended as the optimal loading density.

Figure D6. Photograph of exposure system with stone density of 4 per 100 cm^2 with sediment for methods development of Upper Columbia River sediment toxicity tests.

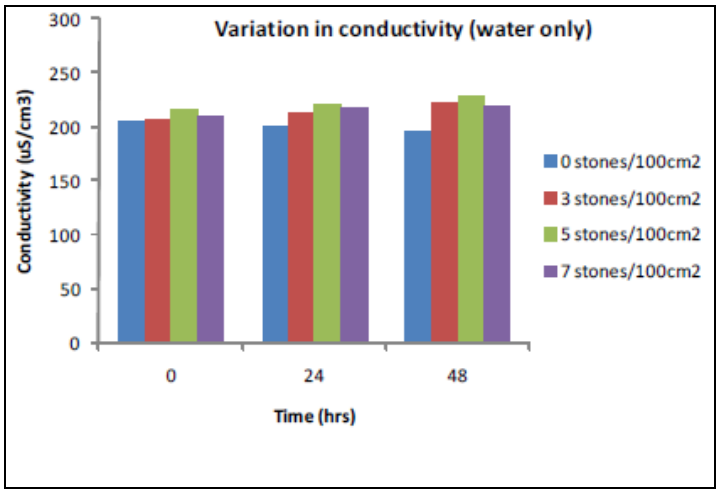


Figure D7. Variation in conductivity in water-only exposure systems over 48 hrs at stone densities of 0, 3, 5 and 7 stones per 100 cm^2 for methods development of Upper Columbia River sediment toxicity tests.

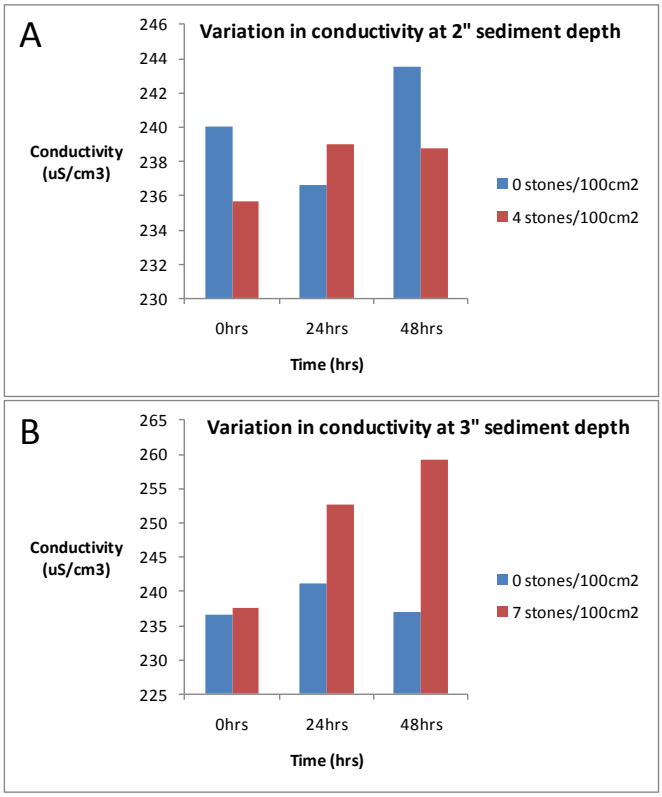


Figure D8. Variation in conductivity at 2" sediment depth over 48hrs with 0 and 4 stones per 100cm^2 (A) and variation in conductivity at 3" sediment depth over 48hrs with 0 and 7 stones per 100cm^2 (B) for methods development of Upper Columbia River sediment toxicity tests.

Order 5 & 6: Assessment of pore water sampling techniques and sediment depth using suction devices

Objective: Establish optimum methods and conditions for sampling pore water.

Experimental Design

1. Pore water sampling device evaluation

Static water only test:

Three different types of suction devices (i.e. airstones; Rena Micro Bubbler 6" [ceramic] cat. #704E; Penn-Plax Aqua-Mist 6" [sandstone] cat. #A6M; Penn-Plax Bubble Wall 10" [paper] cat. #BW10) were tested regarding their utility to sample water by means of suction through a 10 mL syringe. Static, water only tests were conducted by placing airstones in buckets of water and pulling water through the airstone. All tests were done in the same manner. Homogeneity of sampling across the surface of the sampling device was assessed by introducing dye via a 10 mL pipette at different portions (anterior, centre and posterior) parts of the different airstones during application of suction to the device, and by visually assessing presence of dye in the water. Deadspace in airstones were minimized by inserting an acrylic rod into the hollow core. A selection of experiments were video-documented.

2. Evaluation of sediment depth

Static water tests with sediment at various depths using ceramic airstones:

This test was conducted in 10 L aquaria with two laboratory control sediments. Loose gravel (2.0 - 5.0 mm, geosystems substrate, #12418, hagen) was placed into test chambers at

depths of 1” (inch), 2”, and 3”, while finer sediment (1.0 – 2.0 mm, geosystems substrate, #12648, hagen) was placed into test chambers at depths of 1”, 2”, 3”, and 4”. Airstones were placed at the bottom of the aquarium. Dye was introduced to the surface water and the amount of water that could be removed by the airstone before the introduction of dye was recorded. The presence of dye was measured visually by use of a black light.

Flow through tests with sediment at various depths using ceramic airstones:

Sediments were layered at a thickness of 1, 1.5, 2, and 3 inches into the exposure portion of the fluvial test chambers. Up to sixteen pore water sampling ports were equally distributed throughout the exposure chambers at a depth of 1 and 1.5 inches below the sediment surface (Figure D9). This represents a slight deviation from the originally proposed design that asked for testing sediment depths of 2, 3, and 4 inches, and for sampling depths of 0.5 and 1 inch. It was not possible to install sampling devices at a sampling depth of 0.5 inches due to exposure of portions of the sampling device to the overlying water. Also, it was determined as part of the experiment “*Static water tests with sediment at various depths using ceramic airstones”* that sediment depth greater than 3 inches did not proportionally improve overall recoverable sample volumes and resulted in sufficient volumes (>350 mL), and thus, it was decided to focus on the characterization of lesser sediment depth. Furthermore, the experiment using 2 inches of sediment depth was conducted both in absence and presence of gravel in accordance with the density determined in order #4 (4 stones per 100 cm^2). All other initial experiments were conducted without addition of gravel. After introduction of dye into the test system, pore water was sampled until dye was visible in the sampling device (10 mL syringe), and the volume sampled was recorded for each airstone. These initial experiments were followed by a second

series of tests where 2 and 3 inches of sediment were layered into the fluvial test chambers, and which were supplemented with gravel as described above and in order #4. In these experiments airstones were installed at a depth of 1.5 inches, and after the introduction of dye pore water was sampled as described for the initial experiment with the difference that in addition to the visual assessment a sub-sample of the extracted pore water was subjected to fluorescence determination by use of a microtiter plate fluorescent reader (Polastar Optima, BMG Labtech, Offenburg, Germany). Samples for dye concentration determination were collected at 10 mL intervals up to 110 mL of total volume sampled (= 11 samples). All experiments described within this section were conducted at flow rates of 25 L/min.

Decision Criteria

Descriptive stats (means +/- SD). Comparison of mean values using parametric statistics (Student's t-test). Visual assessment of the presence of fluorescence based on video records and other visual documentation, as appropriate.

Results

1. Pore water sampling device selection

Static water only test:

Of the three airstones tested the Plax Aqua-Mist 6" (sandstone) did not allow for pulling significant amounts of water (≥ 30 mL). While it was possible to retrieve the required amounts of water (by suction from the Penn-Plax Bubble Wall 10" (paper) device, this airstone had a strong preference for pulling water from the front ½ of the airstone's length as demonstrated by the dye

test. The ceramic airstone (Rena Micro Bubble) appeared to pull water evenly along the whole surface of the airstone, and therefore, was selected for further evaluation. All assessments were made by means of visual inspection of the presence or absence of dye.

2. Selection of sediment depth

Static water tests with sediment at various depths using ceramic airstones:

Volumes of pore water that could be sampled without incorporation of overlying water increased significantly ($p=0.001$) with sediment depths greater than 1 inch. 20 mL of pore water was retrieved in 1.0 inch of loose gravel sediment depth compared to 130 mL in 2.0 inch of loose gravel sediment depth. 230 mL of pore water was retrieved in 3.0 inch of loose gravel sediment depth. Greater pore water volumes were sampled in equivalent depths of the fine sediment and averages ranged from 30 mL pore water retrieved in 1.0 inch of fine sediment depth compared to 193 mL in 2.0 inch of fine sediment depth. Average volumes of 320 mL and 870 mL of pore water were retrieved from 3.0 inch and 4.0 inch depths of fine sediment, respectively.

Flow through tests with sediment at various depths using ceramic airstones:

Volumes of pore water that could be retrieved without incorporation of overlying water were dependent on the depth of airstone installment rather than overall sediment depth (Figure D10). Minimum retrievable volumes per sampling port ranged from 15 mL at a sediment depth and sampling depth of 1 inch; to 100 mL at a sediment depth of 3 inches and a sampling depth of 1.5 inch (Figure D11). The minimum and average sample amounts that could be retrieved at a sampling depth of 1.5 inch were 73 and 107 mL/port, respectively. Furthermore, there was an

influence of the number of ports sampled on the retrievable pore water volume (Figure D12). When only 8 ports were sampled the average retrievable pore water amount was 125 mL/port, while the retrievable amount was almost proportionally and significantly reduced when 16 ports were sampled (79 mL/port; p=0.003). However, total number of ports had no influence on the total retrievable volume from all ports (p=0.133).

To verify the above visually determined retrievable volumes an objective assessment of dye concentrations was conducted by means of fluorescence quantification using the Polastar Optima microtiter plate fluorescent reader. This experiment indicated that volumes that could be retrieved without incorporation of dye from the overlying water were 50 and 60 mL/port for sediment depths of 2 and 3 inches, respectively (Figure D13). Exceptions were ports 1 or 16, which are located directly at the inflow and outflow of the test chambers. If ports 1 and 16 were included, these volumes were reduced to 30 and 40 mL/port for the 2 and 3 inch sediment depths, respectively.

Conclusions:

Based on the data it was recommended to utilize a sampling depth of 1.5 inches due to the enhanced sampling properties as defined by significantly large retrievable volumes. Because depth of sediments did not significantly affect pore water sampling, and considering volume restrictions for certain sediments, it was proposed to use a seeding depth of 2 inches in the definitive exposure study. This study did not permit assessment of the influence of the removed sediment volumes on re-equilibration of pore water but it was assumed that the here described maximum volumes will significantly deplete pore water. Therefore, it was recommended to

reduce the least amounts of pore water to a third of the minimum amount reported during the objective assessment of dye concentrations (fluorometer measurement) that could be removed without detection of dye from overlying water, namely 15 and 8 mL per port using a design of 8 or 16 ports, respectively. Also, given some of the variation observed at the ports located closest to the in- and outflow of the test chambers, it was proposed not to sample within the first and last 4 inches of the fluvial test systems. To insure that during a sampling event there was equal drawing of pore water throughout the sediment layer, a minimum of 8 ports was sampled, at approximately 8-10 mL each, resulting in a total sample volume of approximately 80 mL, which was sufficient for the water quality and metals analyses in the white sturgeon definitive sediment toxicity study.

Figure D9. Distribution of airstones throughout the test chamber (shown without sediment) for methods development of Upper Columbia River sediment toxicity tests.

The example presented here has 8 airstones installed. The external view (B) shows sampling ports through which pore water would be removed.

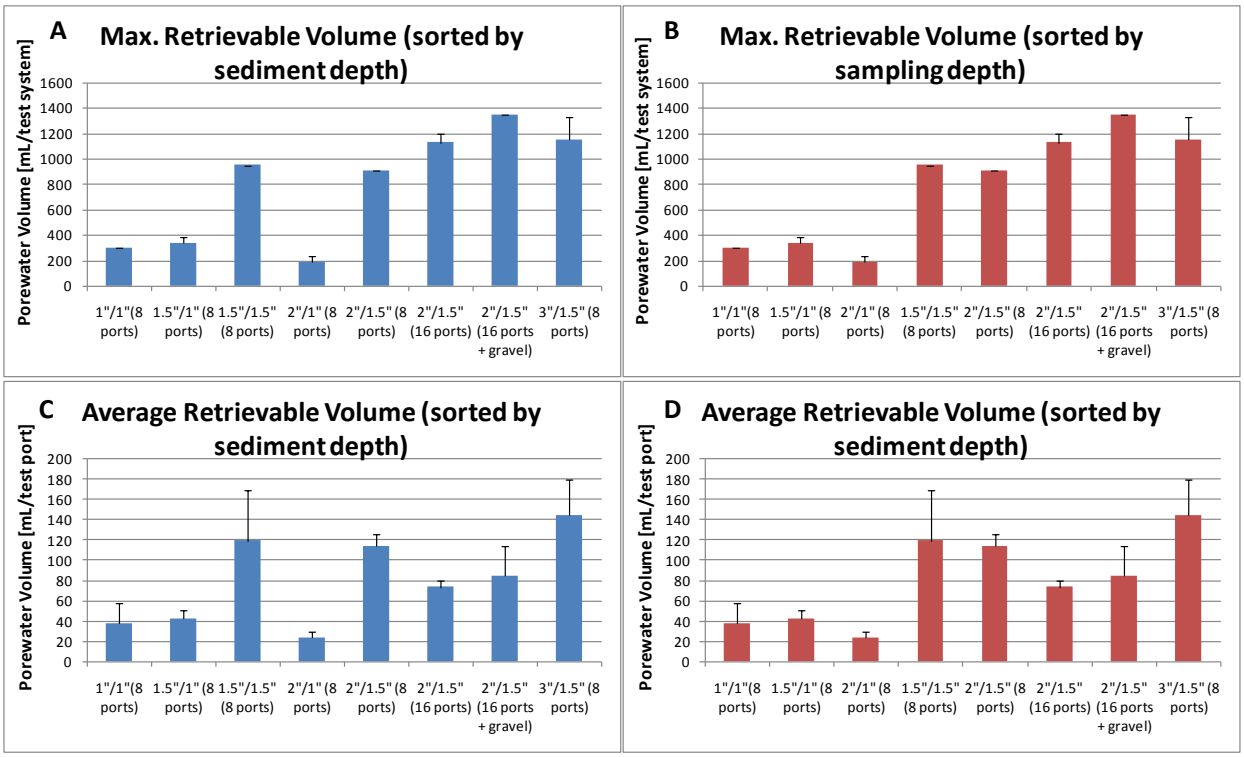


Figure D10. Mean maximum recoverable volumes of pore water per test system (A&B) and average recoverable volumes per individual sampling port (C&D) under different sampling regimes and sediment depths for methods development of Upper Columbia River sediment toxicity tests.

Data was sorted by sediment depth (A&C) and depth of sampling device (B&D). Bars represent mean values ± 1xSD (error bars). X-axis numbers represent sediment (first number) and pore water sampling (second number) depths in inches. The numbers in brackets after the sediment and pore water sampling depth indicate the number of ports that were sampled during each experiment.

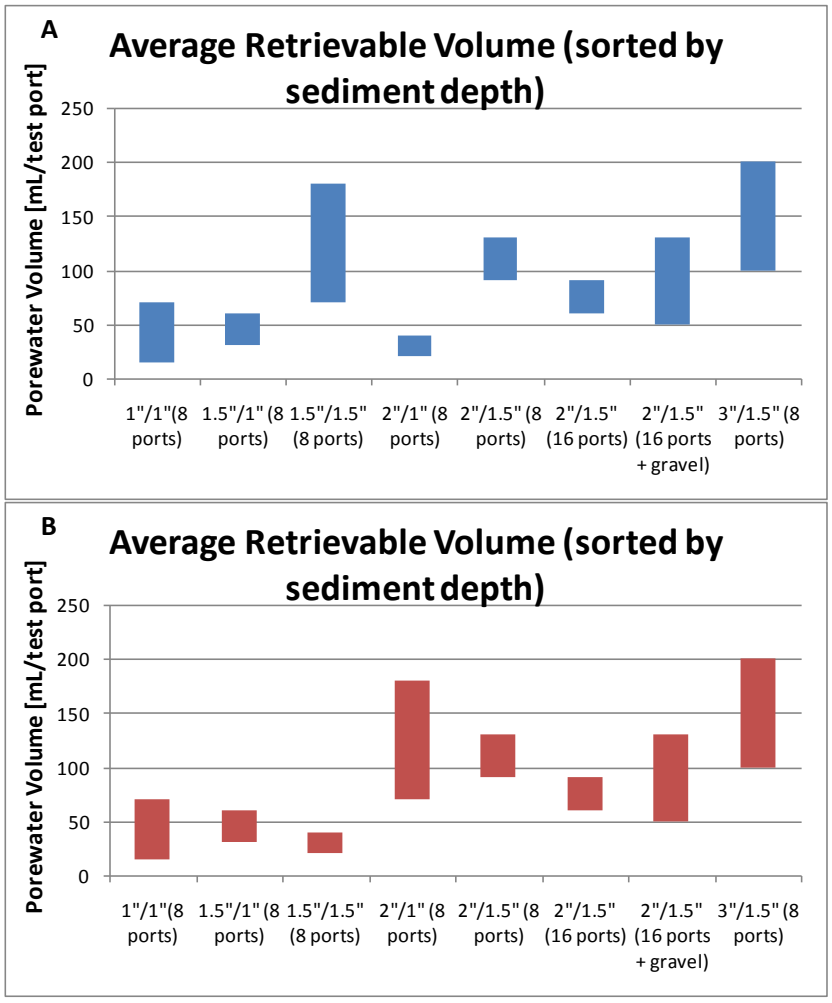


Figure D11. Minimum-maximum ranges for retrievable volumes of pore water per sampling port under different sampling regimes and sediment depths for methods development of Upper Columbia River sediment toxicity tests.

Data was sorted by sediment seeding depth (A) and depth of sampling device (B). Upper and lower boundaries of bars define maximum and minimum ranges for sample volumes that could be retrieved without ingestion of dye from overlying water.

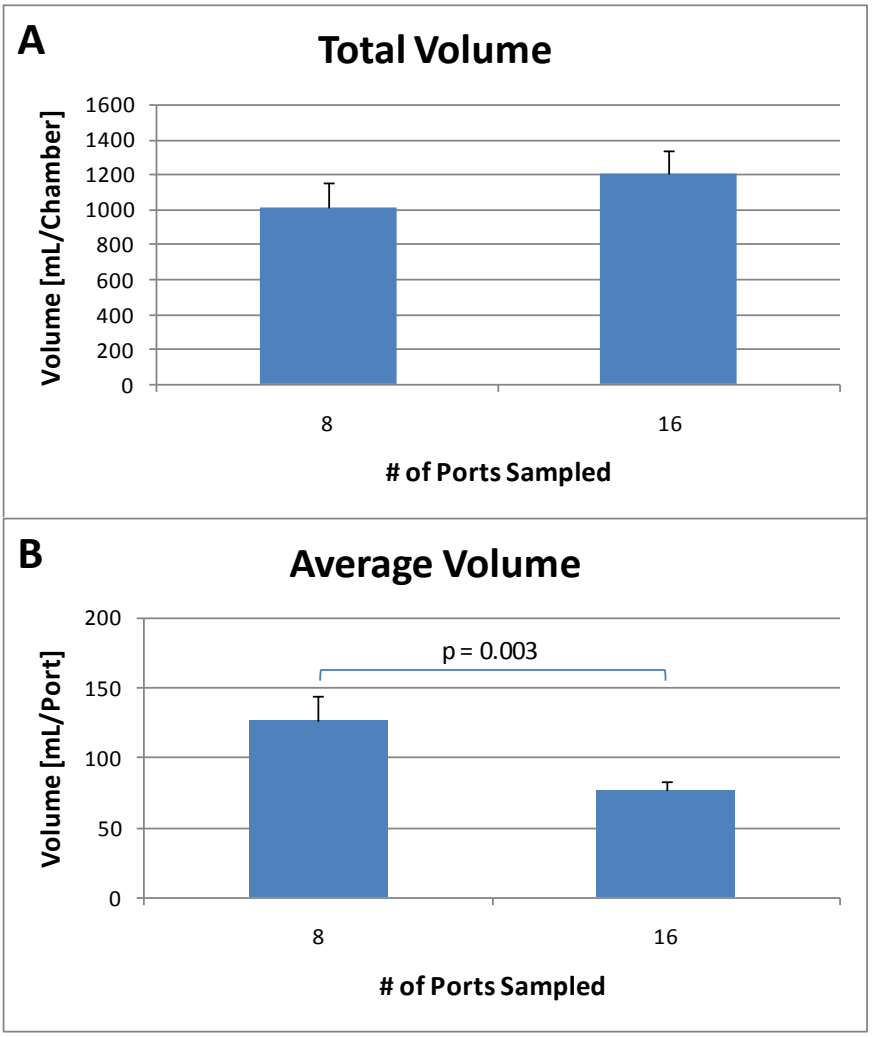


Figure D12. Average sample volumes per test system (A) and per sampling port (B) that could be retrieved without ingestion of dye from overlying water as a function of the total number of ports used per test chamber for methods development of Upper Columbia River sediment toxicity tests.

Bars represent mean values ± 1xSD (error bars). Statistical test: Student's t-test (pair-wise comparison of means).

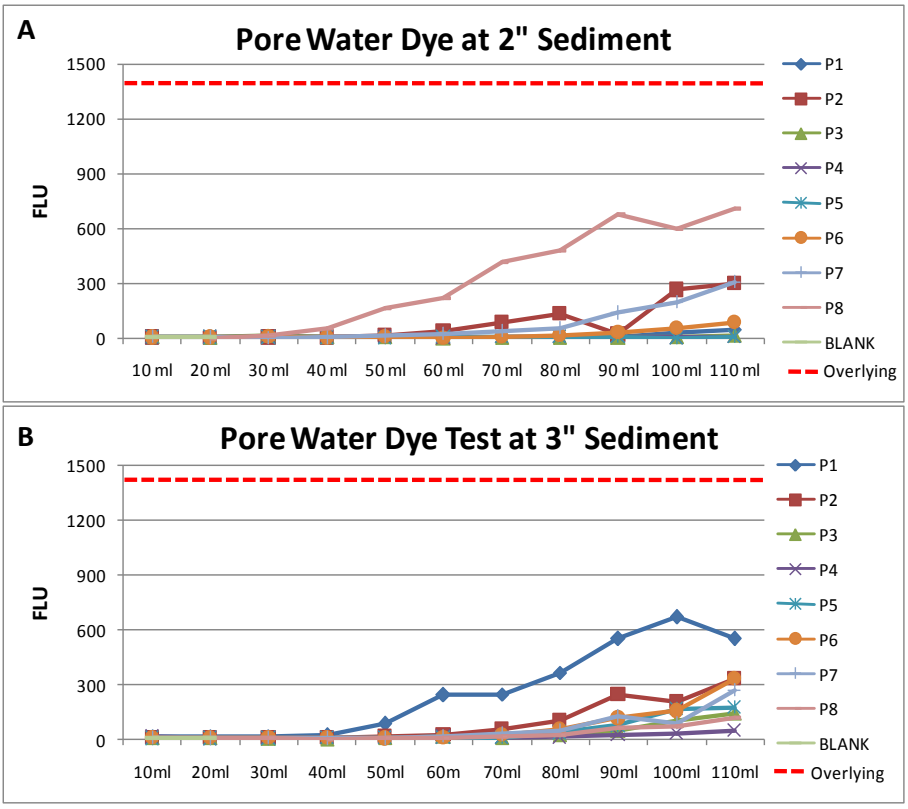


Figure D13. Fluorescence intensities (FLUs) measured in pore water removed from sampling ports 1 through 8 (P1 – P8) at 10 mL sampling intervals after introduction of dye into overlying water for methods development of Upper Columbia River sediment toxicity tests.

Experiments were conducted at sediment seeding depth of 2 (A) and 3 (B) inches in the test chamber, and pore water was sampled at a depth of 1.5 inches beneath the sediment surface. Red dotted line: Average dye concentration in overlying water.

Order 7: Gradients between pore- and overlying water

Objective: Evaluate potential gradients between pore water and overlying water under different

hydrological conditions (e.g., flow velocity).

Experimental Design

Gradients in conductivity, pH and dissolved organic carbon (DOC) between pore water and overlying water were measured by means of suction devices (airstones) that were installed at 10.2 cm intervals along the entire length of the centre of the sediment exposure chamber. Suction devices were buried at different depths to enable sampling of pore water in the top 2.5 cm of sediment and in the sediment-surface water transitional zone (pseudo hyporheic area with 4 rocks per 100 cm^2 for habitat enrichment as described in Order #4). Experiments without rocks for habitat enrichment were excluded as it was decided based on the findings from Order 2 that rocks were to be used in subsequent sturgeon experiments. Parameters that were used to assess potential gradients between pore water and overlying water were conductivity, DOC and pH. Measurements were made 24, 48, and 96 hr after initiation of the experiment. Dye was not used in these experiments since it was discovered during studies for Orders 5 and 6 that dye would not readily seep into the sediment without being physically pulled through. Flows that were tested ranged from greater (25 L/min) to lesser flow rates (17 L/min). These flow-rates were deemed appropriate for maintaining conditions appropriate for white sturgeon early life stage culture.

Decision Criteria

The goal of this experiment was to establish conditions under which the gradient between pore water and overlying water in the pseudo-hyporheic area is minimal while maintaining conditions appropriate for sturgeon early life stage culture.

Results

There were significant differences between overlying water and pore water for a number of parameters (Figures D14-16). These differences were most prominent for conductivity where significantly greater values were recorded in pore water samples regardless of depth and time of sampling (Figure D14). There were differences in conductivity between pore water at different greater depths in the sediment. However, the differences were less than those between overlying water and pore water. Also, in the reference sediment treatment group (Genelle sediment) statistically significant increases in conductivity in pore water were observed between the 24 and 96 hr at the greatest sampling depth. No such differences occurred in the experiment with Deadman's Eddy (DE) sediment. Flow rate did not have an effect on conductivity in any of the matrices analyzed.

In the reference sediment experiment there was a statistically significant decrease in pH of pore water from both depths relative to that in overlying water. No such difference was observed in the DE sediment test group. In fact, pH was not different among sampling times or depths in this group. In general, DOC concentrations were highly variable in pore water when measured 24h after initiation of the experiment. It is assumed that these differences between the early (24h) and later measurements are due to the fact that the DE substrate tested was of a dry nature prior to submersion in the test systems, and therefore, at 24h there were still significant dissolution processes ongoing. Similarly, the reference sediment (saturated with water during storage) used was mixed and introduced into the test system just prior to t=0, likely resulting in a very different initial pore water composition that slowly mixed with overlying water until a certain degree of steady-state was reached. It is assumed that after 48h this dissolution or

exchange between overlying and pore water was mostly completed or had stabilized. While the reference sediment group showed no further change in pore water DOC concentrations after 48h, there was still an apparent decrease in DOC concentrations in the DE sediments after 96h. It is not possible, however, to extrapolate from this observation to riverine sediments because the DE substrate was dry (collected above the water line from a beach/gravel bar). It can be assumed that water saturated sediments will behave very differently due to the lack of initial dissolution processes. This may also explain the differences observed between the DE and reference substrate. Depth of pore water sampling did not have a marked effect on DOC patterns.

Conclusions

There were gradients among measurement parameters between overlying water and pore water. These appeared, however, to not be influenced by flow-rate or duration with the exception of a small difference in the reference sediment at the greatest pore water sampling depth. This also indicates - with the exception of some sediments at greater sampling depth - that gradients were relatively stable and do not change over time under constant flow-conditions. Also, the time-depended increase in conductivity at greater sampling depth as observed for the reference sediment could be indicative of shallower sediment horizons reaching steady-state more quickly. The reason why this only occurred for the reference substratum could be due to the fact that the DE sediment was collected from the gravel bar above the water line, and thus, may contain lesser amounts of fines. This could result in a lesser porous structure of the reference sediment causing more resistance in the flow of pore water between sediment horizons.

Furthermore, sediment-sampling depth had a significant influence on conductivity but not pH or DOC. Based on this result and the findings from Orders 5 and 6, to enable sampling of sufficient volumes while reducing differences between overlying water and pore water sampling ports at shallower sampling depths between 2.5 and 3.4 cm was recommended. Also, considering the lack of time-dependency of gradients between overlying water and pore water, indicating rapid establishment of steady-state after initiation of the experiments, it was favoured to reduce the equilibration time prior to introduction of test organisms in the definite exposure studies.

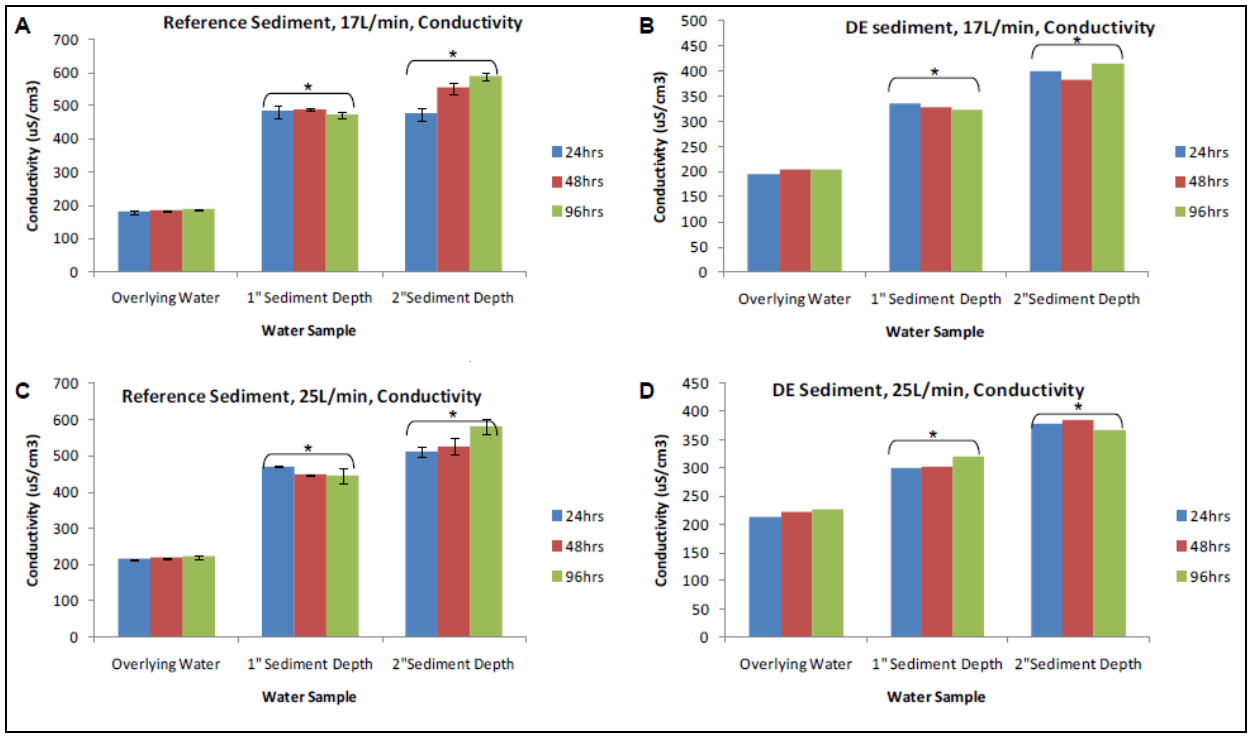


Figure D14. Mean conductivity in overlying water and pore water at flow-rates of 17 (A&B) and 25 (C&D) L/min for methods development of Upper Columbia River sediment toxicity tests.

Overlying and pore waters were sampled at depths of 1 and 2 inches at 24, 48 and 96 h after initiation of experiment. Sediment types tested were reference sediment and sand bar substrate collected at Genelle (A&C) and Deadman's Eddy (B&C). Asterisks indicate significant difference from mean response in overlying water measured at the same time ($p<0.05$; Student's t-test).

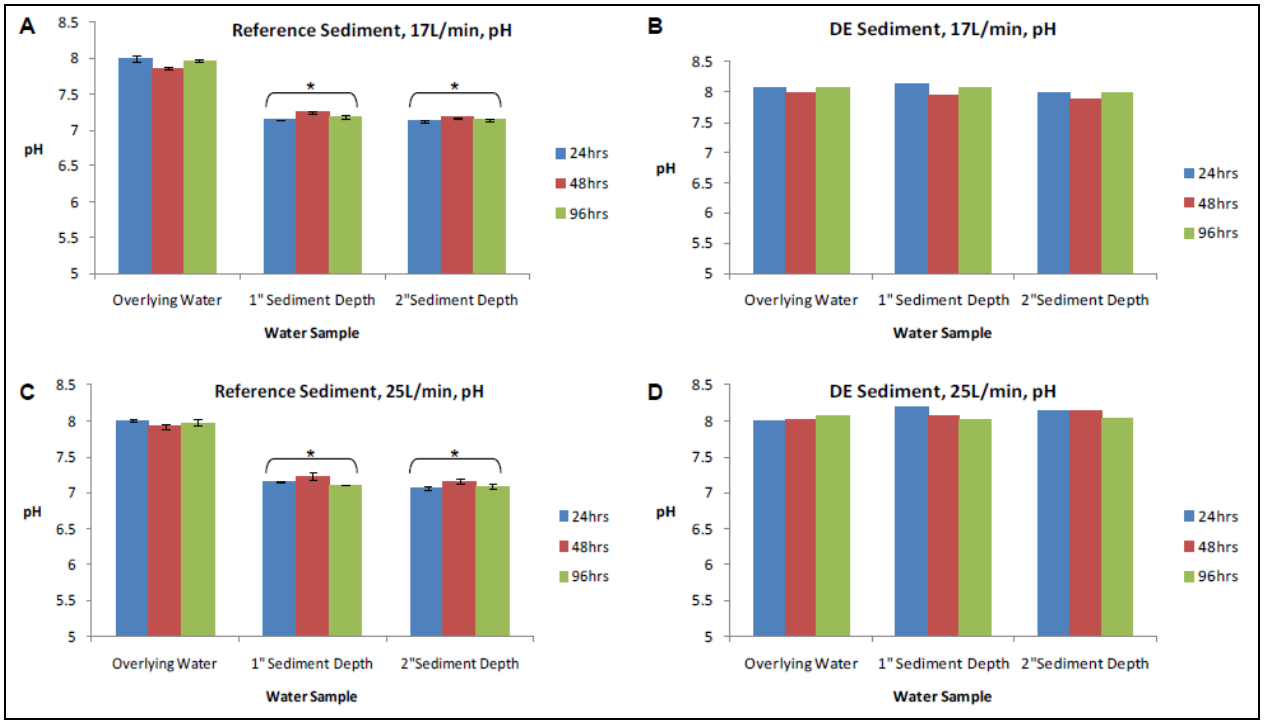


Figure D15. Mean pH in overlying water and pore water at flow-rates of 17 (A&B) and 25 (C&D) L/min for methods development of Upper Columbia River sediment toxicity tests.

Overlying and pore waters were sampled at depths 1 and 2 inches at 24, 48 and 96 h after initiation of experiment. Sediment types tested were reference sediment and sand bar substrate collected at Genelle (A&C) and Deadman's Eddy (B&C). Asterisks indicate significant difference from mean response in overlying water measured at the same time ($p<0.05$; Student's t-test).

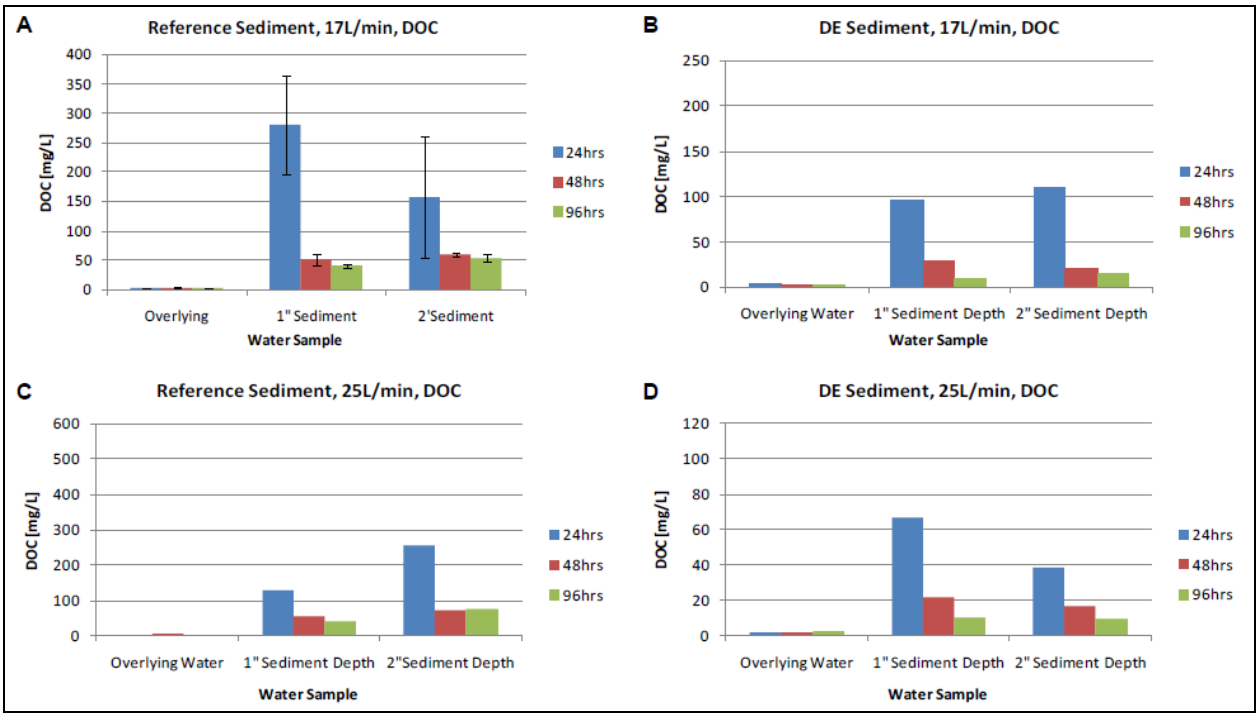


Figure D16. Mean DOC [mg/L] in overlying water, and pore water at flow-rates of 17 (A&B) and 25 (C&D) L/min for methods development of Upper Columbia River sediment toxicity tests.

Overlying and pore waters were sampled at depths of 1 and 2 inches at 24, 48 and 96 h after initiation of experiment. Sediment types tested were reference sediment and sand bar substrate collected at Genelle (A&C) and Deadman's Eddy (B&C). Asterisks indicate significant difference from mean response in overlying water measured at the same time ($p<0.05$; Student's t-test).

Order 8: Time to 'steady-state' after introduction of test sediments into the test chambers

Objective: The objective was to identify the minimum period of time necessary for the exposure chamber to attain a steady-state based on basic water quality parameters. The objective of this work was not to attain steady-state conditions for chemicals of potential concern (COPCs); but rather, to ensure that non-COPCs do not adversely affect test results (i.e., introduce uncertainty) when organisms are introduced.

Experimental Design

Basic water quality parameters were monitored in test chambers containing river sediment at 0, 12, 24, and 48 h, and every 48 h thereafter until steady state. Measurements included conductivity, dissolved oxygen (DO), ammonia, nitrate, colour, total dissolved solids (TDS), and pH at the inflow and outflow of the exposure chamber.

Decision Criteria

'Steady-state' is attained when measured water quality parameters do not vary more than 10 percent from one measurement event to the next.

Results

Over the course of the experiment values for temperature, dissolved oxygen, pH, and colour had no significant variability (greater than ± 10 percent between measurement events) at either the inflow or outflow of the test chamber. Values for ammonia and nitrate had significant variability at both the inflow and outflow of the test chamber between measurement events. The measurements for conductivity, total dissolved solids (TDS), and dissolved organic carbon (DOC) are considered the most likely parameters to determine steady-state. Conductivity and TDS had no significant variability during the experiment. DOC did have variability during the experiment with measurements as high as 41.67 % between the readings at 48 and 96 hours at the inflow and 21.43 % at the outflow.

Conclusions

Steady-state is achieved for temperature, conductivity, dissolved oxygen, pH, colour, and total dissolved solids within 48 hours. Ammonia and nitrate did not reach steady-state, but this is likely due to the natural variability of these measurements. DOC did not reach steady-state and after further experimentation it is suspected that DOC may not reach steady-state.

Order 9: Determination of most efficient cleaning method minimizing re-suspension of sediments.

Objective: Identify optimum cleaning techniques without utilization of invasive suction devices while employing large particle filters-with and without addition of diet (bloodworms, oligochaetes, semi-moist diet, and *Artemia*).

Experimental Design

Food was introduced simulating three feeding event per day using *Artemia*, worms and semi-moist diet. At days 2, 3, 4, and 5 chambers were manually cleaned (daily) to remove as much biofilm as possible without significant re-suspension of sediments. At 5, 10, 20, and 30 minutes after each cleaning event, near bottom water samples, approximately 1 cm above the sediment surface, were sampled. Turbidity of samples as a measure of re-suspended matter was determined using light scattering methods as described in EPA Method 180.1 or Standard Method 2130B (Standard Method 1995). Three different cleaning techniques were initially investigated at the beginning of the experiment:

1.) Siphoning the sediment surface with the use of a 3/8” ID hose.

2.) Scraping the sediment surface with the use of a plastic spatula.

3.) Pipetting debris with the use of a modified pipette.

After initial attempts it was decided that siphoning and scraping with a spatula were not appropriate methods of cleaning sediment surfaces as they were too invasive and ineffective, respectively.

Decision Criteria

Optimum cleaning techniques were determined as a function of minimizing re-suspension of sediment and efficiency of cleaning as determined by measurements of turbidity. It is acknowledged that any type of physical removal of bio-growth will cause re-suspension to a certain degree, and the final method to be established will be a compromise between efficiency of cleaning and amount of sediment re-suspended during the cleaning event.

Results

1. Siphoning

Siphoning debris from the sediment surface was effective but was deemed too invasive as it removed and disturbed sediment in the process. Grains of sediment were removed from the chamber in the cleaning process. Past experience demonstrated an increased risk of fish injury as some organisms would be sucked into the cleaning tube. Turbidity analyses did not reveal any significant differences between pre-cleaning conditions or at any time period of up to 30 minutes

post cleaning (Figure D17 A). Investigation of siphoning as a cleaning method was discontinued after day 2.

2. Scrapping with a spatula

Scrapping with a spatula was ineffective. It was time consuming and once the debris was dislodged from the sediment surface it was difficult to remove from exposure. Turbidity analyses revealed an increase in turbidity 5 and 10 minutes post cleaning and decreasing there after (Figure D17 B). Investigation of scrapping with a spatula as a cleaning method was discontinued after day 2.

3. Modified pipette

Pipetting the sediment surface to remove debris proved to be the most efficient and less invasive method. Biofilm and food could be easily dislodged from the sediment surface with minimal disturbance to sediment and effectively removed from the exposure chamber. Turbidity analysis revealed no significant differences between pre-cleaning conditions or at any time period of up to 30 minutes post cleaning for the entirety of the experiment (day 2 -5), with the one exception at day 3, ten minutes post cleaning (Figure D17 C). Turbidity analyses at this time point revealed 0.6 NTU. This is considered to be a condition of other factors than cleaning techniques as all other turbidity results were within normal ranges throughout the 30 minute test. The possible introduction of foreign material during sampling could explain elevated turbidity levels at this time point.

Conclusions

Cleaning by use of a modified pipette allowed the technician to select unwanted debris and remove it from the exposure chamber with minimal disturbance to the sediment. In addition, the risk of injury to fish was minimized. Siphoning proved effective when cleaning reservoirs or screens, but only when direct contact with fish and sediment was not involved. The modified pipette was used as the primary cleaning method for sediment within the exposure chamber.

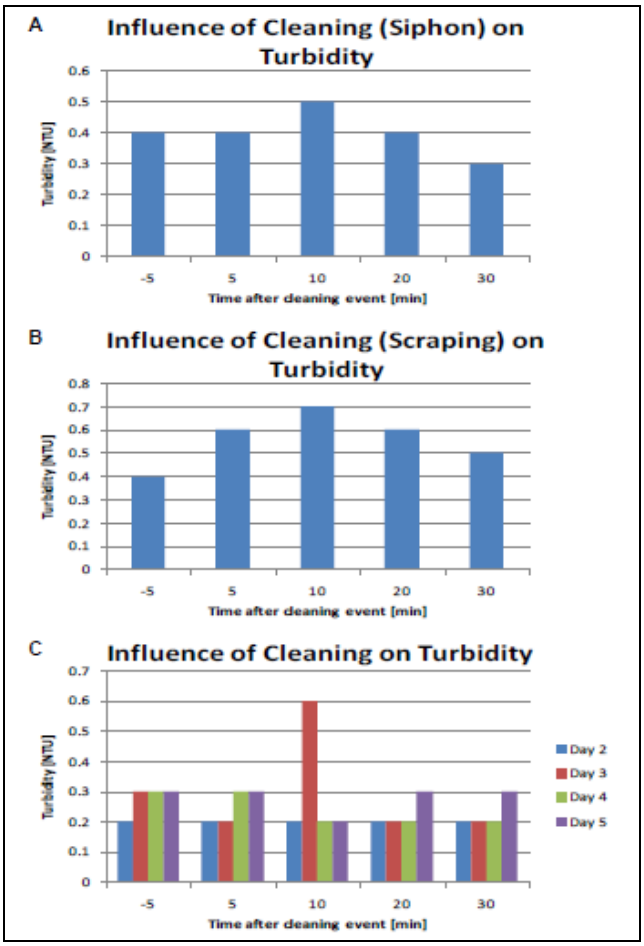


Figure D17. Turbidity in surface water prior (-5 [minutes]) and after (5, 10, 20 and 30 [minutes]) addition of food to fluvial test chamber at days 2 (A, B, C), 3 (C), 4(C) and 5(C) after initiation of feeding routine as determined by nephelometry using different cleaning techniques (A: Siphoning; B: Scraping; C: Modified Pipette) for methods development of Upper Columbia River sediment toxicity tests. NTU = Nephelometric Turbidity Units (NTU).

Order 10: Artificial laboratory control sediment

Objective: The objective was to select laboratory control sediment that had physical characteristics suitable for early life stages of sturgeon, was similar to sediments found in the UCR, and comparable to sediments used historically in standard early life stage tests with fishes.

Experimental Design

Several different silica sand and crushed/ground granites available from commercial vendors were considered. Sediments were layered into the test chamber and water quality parameters were assessed in comparison to average reference riverine sediment water quality parameters.

Decision Criteria

Criteria used to select suitable laboratory reference sediment were:

• Grain sizes between 0.5 and 2 mm in diameter

• Color similar to UCR sediments (preference for dark coloration)

• pH, dissolved oxygen, hardness, and alkalinity do not differ by more than 50 percent from average values determined for riverine sediments

• Certificate of analysis for contamination

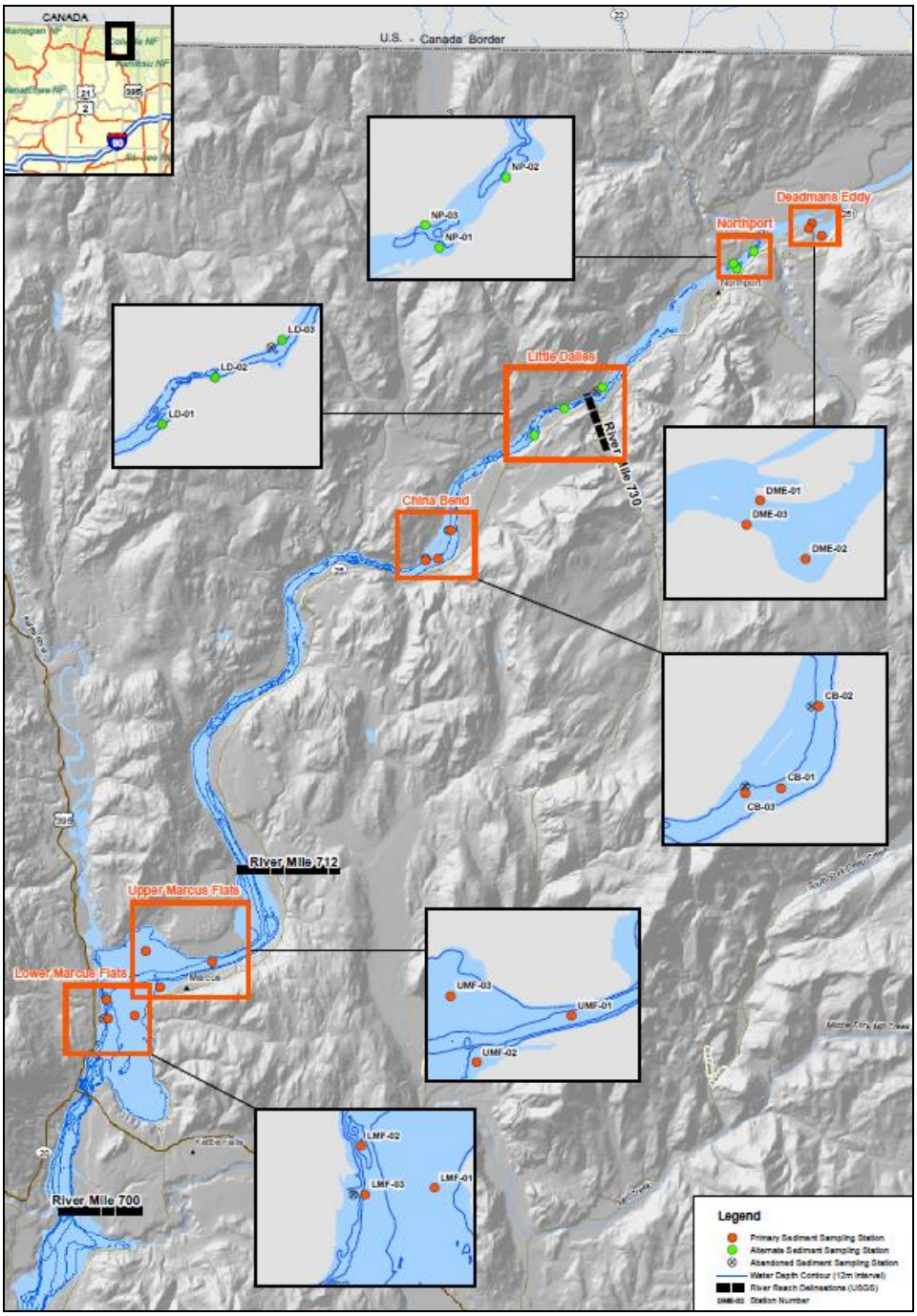
Results

Several different candidate sediments were selected including: Hagen Geosystem Black Fine Gravel (ART# 12648), Hagen Geosystem Extra Fine White River Gravel (ART# 12647), Hagen Geosystem Pacific Gravel (ART# 12404), Hagen Geosystem Black Beach Gravel (ART# 12418), Aquaterra Black Sand (#80035), Aquaterra Natural Tan Sand (#80075), and Pure Water Pebbles Cumberland River Gems (#30095). The Hagen Geosystem Black Fine Gravel (ART# 12648) was the only sediment that met both the grain size and color criteria and so was selected for further trials. No certificate of analysis for contamination was available from the manufacturer so samples of the Black Fine Gravel were analyzed at Columbia Analytical Services (CAS). The Hagen Geosystem Black Fine Gravel did not exceed any of the screening ecological values (SEV; from the SLERA) for chemicals/metals analyzed. The Black Fine Gravel did not change water quality parameters in any significant (± 50%) way compared to average water quality parameters found in test chambers with reference riverine sediments. Upon analysis for percent gravel and percent sand, it was found that the two reference sediments (Genelle and Lower Arrow Lake) were gravelly sand with approximately 25% gravel content. Analysis by CAS showed that none of the chemicals/metals analyzed exceeded SEVs in the artificial control sediment.

Conclusions

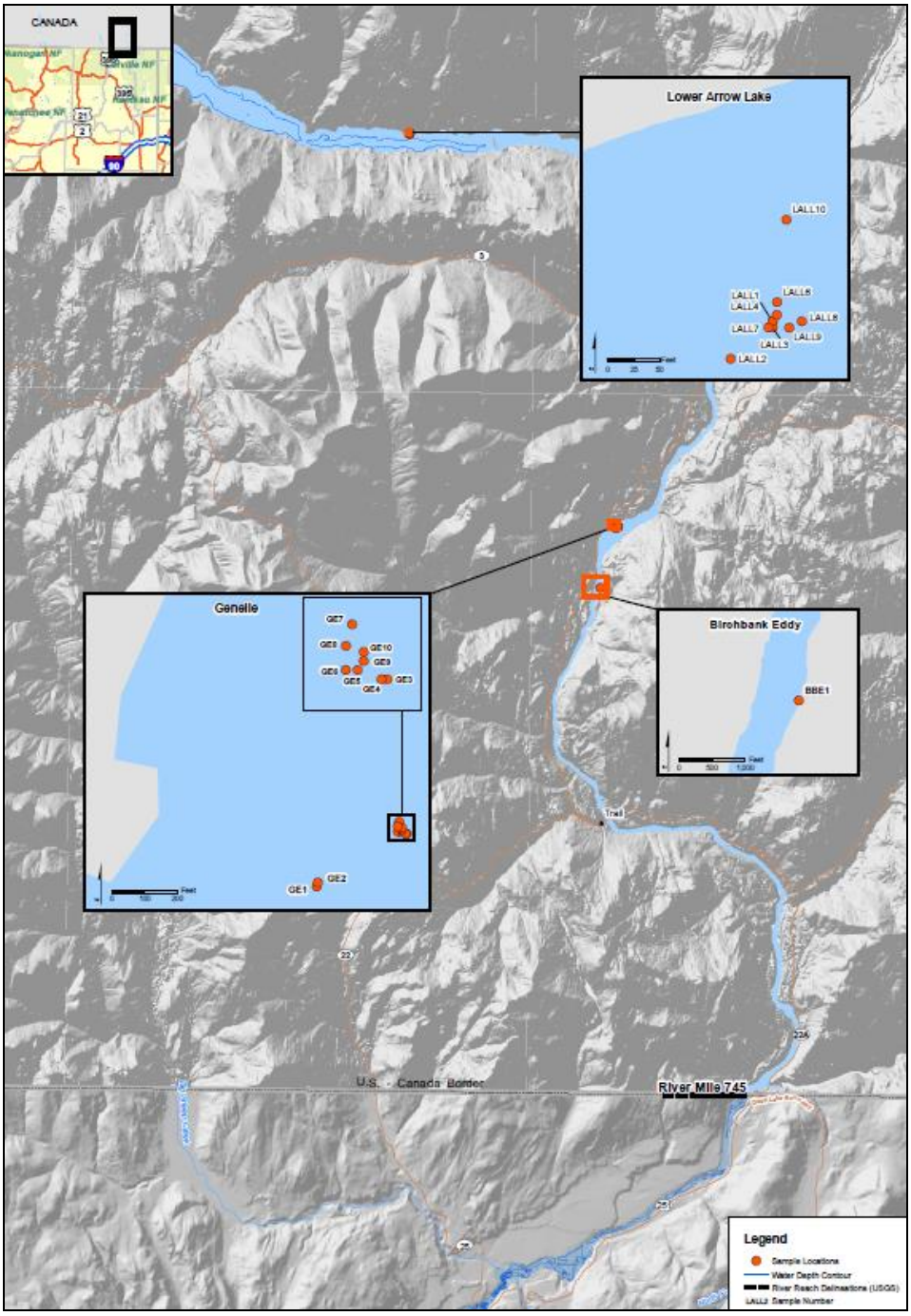
The Hagen Geosystem Black Fine Gravel (ART #12648) was suitable laboratory reference control sediment. This sediment was within the 0.5 to 2 mm grain size with an average grain size of 1.11 mm and a least and greatest grain size of 0.85 mm and 1.68 mm, respectively.

This sediment was predominantly dark in coloration and very similar in appearance to some riverine sediments of interest. This sediment did not change water quality parameters more than 10% compared to the average water quality parameters measured in test chambers with riverine sediments. Analysis by CAS revealed this sediment to be free of contamination by all chemicals/metals analyzed.



Map D1. Map of sites selected for sediment sampling for laboratory exposure studies with early life stage white sturgeon.

Sampling areas included Deadman's Eddy (DME; river mile [RM] 737), Northport (NP; RM 735), Little Dalles (LD; RM 729), China Bend (CB; RM 723), Upper Marcus Flats (UMF; RM 706), and Lower Marcus Flats (LMF; RM 704) in Washington State, USA.



Map D2. Map of reference sediment locations for laboratory exposure studies with early life stage white sturgeon.

Sampling areas included Birchbank Eddy (BBE; river mile [RM] 764), Genelle (GE; RM 766), and Lower Arrow Lakes (LALL; RM 7880) British Columbia, Canada.

Table D3. Sediment analytical methods and associated method detection limits and method reporting limits.

Analyte Type	Analyte	Analytical Method	Units	MDL	MRL
Conventionals					
	Organic carbon	D412982M	percent	0.02	0.05
	pH	EPA 9045D	SU	--	--
	Solids	EPA 160.3	percent	--	--
	Sulfide-AVS	EPA 821 R91-SEM	µmol/g	0.003 - 1.2	0.016 - 0.72
Grain Size					
	Clay	PSEP	percent	--	--
	Coarse Gravel	PSEP	percent	--	--
	Coarse sand	PSEP	percent	--	--
	Cobbles	PSEP	percent	--	--
	Fine Gravel	PSEP	percent	--	--
	Fine Sand	PSEP	percent	--	--
	Med. Sand	PSEP	percent	--	--
	Medium Gravel	PSEP	percent	--	--
	Silt	PSEP	percent	--	--
	Very Coarse Gravel	PSEP	percent	--	--
	Very Coarse Sand	PSEP	percent	--	--
	Very Fine Gravel	PSEP	percent	--	--
	Very fine sand	PSEP	percent	--	--
Metals/Metalloids					
	Aluminum	EPA 6010B	mg/kg	0.3 - 4.9	7.8 - 124
	Antimony	EPA 6010C-AVS-SEM	µmol/g	0.0041 - 0.0522	0.0041 - 0.0522
	Antimony	EPA 6020	mg/kg	0.008 - 0.213	0.043 - 0.213
	Arsenic	EPA 6010C-AVS-SEM	µmol/g	0.0013 - 0.17	0.0013 - 0.17
	Arsenic	EPA 6020	mg/kg	0.04 - 0.1	0.38 - 1
	Barium	EPA 6010B	mg/kg	0.05 - 0.74	1.55 - 24.7
	Beryllium	EPA 6010B	mg/kg	0.03 - 0.05	0.08 - 0.12
	Cadmium	EPA 6010C-AVS-SEM	µmol/g	0.00005 - 0.0056	0.00005 - 0.0056
	Cadmium	EPA 6020	mg/kg	0.003 - 0.008	0.015 - 0.04
	Calcium	EPA 6010B	mg/kg	1.6 - 24.7	7.8 - 124
	Chromium	EPA 6010C-AVS-SEM	µmol/g	0.0011 - 0.0122	0.0011 - 0.0122
	Chromium	EPA 6020	mg/kg	0.01 - 0.59	0.15 - 7.83
	Cobalt	EPA 6010B	mg/kg	0.16 - 2.5	0.39 - 6.18
	Copper	EPA 6010B	mg/kg	0.2 - 3.7	0.5 - 7.4
	Copper	EPA 6010C-AVS-SEM	µmol/g	0.0015 - 0.02	0.0015 - 0.02
	Iron	EPA 6010B	mg/kg	0.5 - 8.7	3.1 - 49.4
	Lead	EPA 6010C-AVS-SEM	µmol/g	0.002 - 0.031	0.002 - 0.031
	Lead	EPA 6020	mg/kg	0.005 - 1.08	0.038 - 9
	Magnesium	EPA 6010B	mg/kg	0.2 - 3.7	3.1 - 49.4

Table D3. Sediment analytical methods and associated method detection limits and method reporting limits.

Analyte Type	Analyte	Analytical Method	Units	MDL	MRL
Metals/Metalloids (continued)					
	Manganese	EPA 6010B	mg/kg	0.02 - 0.4	0.16 - 24.7
	Mercury	EPA 7470A-SEM	µmol/g	0.00004 - 0.00008	0.00004 - 0.00008
	Mercury	EPA 7471A	mg/kg	0.002	0.015 - 0.023
	Molybdenum	EPA 6020	mg/kg	0.01 - 0.14	0.04 - 0.14
	Nickel	EPA 6010B	mg/kg	0.06 - 0.1	0.31 - 0.5
	Nickel	EPA 6010C-AVS-SEM	µmol/g	0.002 - 0.043	0.002 - 0.043
	Potassium	EPA 6010B	mg/kg	16 - 235	58 - 940
	Selenium	EPA 6020	mg/kg	0.1 - 0.4	0.8 - 2
	Silver	EPA 6020	mg/kg	0.004 - 0.047	0.017 - 0.047
	Sodium	EPA 6010B	mg/kg	3.1 - 44	31 - 443
	Thallium	EPA 6020	mg/kg	0.002 - 0.044	0.015 - 0.044
	Vanadium	EPA 6010B	mg/kg	0.2 - 3.7	0.8 - 12.4
	Zinc	EPA 6010B	mg/kg	0.2 - 3.7	1 - 24.7
	Zinc	EPA 6010C-AVS-SEM	µmol/g	0.0015 - 0.169	0.0015 - 0.169
Polycyclic Aromatic Hydrocarbons					
	2-Methylnaphthalene	EPA 8270C-SIM	µg/kg	0.46	2.6 - 3.2
	Acenaphthylene	EPA 8270C-SIM	µg/kg	0.59	2.8
	Anthracene	EPA 8270C-SIM	µg/kg	0.58	2.8
	Benzo(e)pyrene	EPA 8270C-SIM	µg/kg	0.61	2.7 - 3.1
	Benzo[a]anthracene	EPA 8270C-SIM	µg/kg	0.72	2.6 - 3
	Benzo[a]pyrene	EPA 8270C-SIM	µg/kg	0.76	2.8 - 3
	Benzo[b]fluoranthene	EPA 8270C-SIM	µg/kg	0.92	2.8 - 3.1
	Benzo[g,h,i]perylene	EPA 8270C-SIM	µg/kg	0.85	2.8 - 3
	Benzo[k]fluoranthene	EPA 8270C-SIM	µg/kg	0.87	2.8
	Chrysene	EPA 8270C-SIM	µg/kg	0.8	2.7 - 3
	Dibenzo[a,h]anthracene	EPA 8270C-SIM	µg/kg	0.8	2.8
	Fluoranthene	EPA 8270C-SIM	µg/kg	0.98	2.6 - 3
	Fluorene	EPA 8270C-SIM	µg/kg	0.61	2.7
	Indeno[1,2,3-cd]pyrene	EPA 8270C-SIM	µg/kg	0.87	2.8
	Naphthalene	EPA 8270C-SIM	µg/kg	1.6 - 6.3	2.6 - 6.3
	Perylene	EPA 8270C-SIM	µg/kg	0.72	2.8
	Phenanthrene	EPA 8270C-SIM	µg/kg	1.4	2.7 - 3.2
	Pyrene	EPA 8270C-SIM	µg/kg	0.76	2.6 - 3.1
Polychlorinated Biphenyls					
	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB 206)	EPA 8082	µg/kg	0.031	0.29 - 0.3
	2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194)	EPA 8082	µg/kg	0.043	0.29
	2,2',3,3',4,4',5,6-Octachlorobiphenyl (PCB 195)	EPA 8082	µg/kg	0.031	0.29
	2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170)	EPA 8082	µg/kg	0.026	0.29

Table D3. Sediment analytical methods and associated method detection limits and method reporting limits.

Analyte Type	Analyte	Analytical Method	Units	MDL	MRL
Polychlorinated Biphenyls (continued)					
	2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 128)	EPA 8082	µg/kg	0.031	0.29
	2,2',3,3',4,5',6,6'-Octachlorobiphenyl (PCB 201)	EPA 8082	µg/kg	0.041	0.29
	2,2',3,3',4,5',6'-Heptachlorobiphenyl (PCB 177)	EPA 8082	µg/kg	0.052	0.29
	2,2',3,3',4,5,6'-Heptachlorobiphenyl (PCB 174)	EPA 8082	µg/kg	0.03	0.29
	2,2',3,3',4,6'-Hexachlorobiphenyl (PCB 132)	EPA 8082	µg/kg	0.075	0.29
	2,2',3,4,4',5,5',6-Octachlorobiphenyl (PCB 203)	EPA 8082	µg/kg	0.039	0.29
	2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	EPA 8082	µg/kg	0.095	0.29
	2,2',3,4,4',5',6-Heptachlorobiphenyl (PCB 183)	EPA 8082	µg/kg	0.081	0.29
	2,2',3,4,4',5'-Hexachlorobiphenyl (PCB 138)	EPA 8082	µg/kg	0.064	0.29
	2,2',3,4,4',6,6'-Heptachlorobiphenyl (PCB 184)	EPA 8082	µg/kg	0.052	0.29
	2,2',3,4',5,5',6-Heptachlorobiphenyl (PCB 187)	EPA 8082	µg/kg	0.047	0.29
	2,2',3,4,5,5'-Hexachlorobiphenyl (PCB 141)	EPA 8082	µg/kg	0.035	0.29
	2,2',3,4',5',6-Hexachlorobiphenyl (PCB 149)	EPA 8082	µg/kg	0.067	0.29
	2,2',3,4',5'-Pentachlorobiphenyl (PCB 97)	EPA 8082	µg/kg	0.053	0.29
	2,2',3,4',5-Pentachlorobiphenyl (PCB 90)	EPA 8082	µg/kg	0.035	0.29
	2,2',3,4,5'-Pentachlorobiphenyl (PCB 87)	EPA 8082	µg/kg	0.038	0.29
	2,2',3,5,5',6-Hexachlorobiphenyl (PCB 151)	EPA 8082	µg/kg	0.043	0.29
	2,2',3,5',6-Pentachlorobiphenyl (PCB 95)	EPA 8082	µg/kg	0.049	0.29
	2,2',3,5'-Tetrachlorobiphenyl (PCB 44)	EPA 8082	µg/kg	0.065	0.29
	2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	EPA 8082	µg/kg	0.038	0.29
	2,2',4,4',5-Pentachlorobiphenyl (PCB 99)	EPA 8082	µg/kg	0.045	0.29
	2,2',4,5,5'-Pentachlorobiphenyl (PCB 101)	EPA 8082	µg/kg	0.049	0.29
	2,2',4,5'-Tetrachlorobiphenyl (PCB 49)	EPA 8082	µg/kg	0.058	0.29
	2,2',5,5'-Tetrachlorobiphenyl (PCB 52)	EPA 8082	µg/kg	0.059	0.29
	2,2',5-Trichlorobiphenyl (PCB 18)	EPA 8082	µg/kg	0.096	0.29
	2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB 189)	EPA 8082	µg/kg	0.029	0.29
	2,3,3',4,4',5'-Hexachlorobiphenyl (PCB 157)	EPA 8082	µg/kg	0.031	0.29
	2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156)	EPA 8082	µg/kg	0.042	0.29
	2,3,3',4,4',6-Hexachlorobiphenyl (PCB 158)	EPA 8082	µg/kg	0.028	0.29
	2,3,3',4,4'-Pentachlorobiphenyl (PCB 105)	EPA 8082	µg/kg	0.033	0.29
	2,3,3',4',6-Pentachlorobiphenyl (PCB 110)	EPA 8082	µg/kg	0.035	0.29
	2,3,3',4'-Tetrachlorobiphenyl (PCB 56)	EPA 8082	µg/kg	0.046	0.29
	2,3',4,4',5,5'-Hexachlorobiphenyl (PCB 167)	EPA 8082	µg/kg	0.046	0.29
	2,3',4,4',5',6-Hexachlorobiphenyl (PCB 168)	EPA 8082	µg/kg	0.027	0.29
	2,3,4,4',5,6-Hexachlorobiphenyl (PCB 166)	EPA 8082	µg/kg	0.03	0.29
	2,3',4,4',5'-Pentachlorobiphenyl (PCB 123)	EPA 8082	µg/kg	0.067	0.29
	2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	EPA 8082	µg/kg	0.031	0.29
	2,3,4,4',5-Pentachlorobiphenyl (PCB 114)	EPA 8082	µg/kg	0.023	0.29

Table D3. Sediment analytical methods and associated method detection limits and method reporting limits.

Analyte Type	Analyte	Analytical Method	Units	MDL	MRL
Polychlorinated Biphenyls (continued)					
	2,3',4,4'-Tetrachlorobiphenyl (PCB 66)	EPA 8082	µg/kg	0.035	0.29
	2,3,4,4'-Tetrachlorobiphenyl (PCB 60)	EPA 8082	µg/kg	0.039	0.29
	2,3',4',5-Tetrachlorobiphenyl (PCB 70)	EPA 8082	µg/kg	0.051	0.29
	2,3',4'-Trichlorobiphenyl (PCB 33)	EPA 8082	µg/kg	0.11	0.29
	2,3-Dichlorobiphenyl (PCB 5)	EPA 8082	µg/kg	0.13	0.29
	2,4,4',5-Tetrachlorobiphenyl (PCB 74)	EPA 8082	µg/kg	0.044	0.29
	2,4,4'-Trichlorobiphenyl (PCB 28)	EPA 8082	µg/kg	0.064	0.29
	2,4',5-Trichlorobiphenyl (PCB 31)	EPA 8082	µg/kg	0.056	0.29
	2,4'-Dichlorobiphenyl (PCB 8)	EPA 8082	µg/kg	0.21	0.29
	2-Chlorobiphenyl (PCB 1)	EPA 8082	µg/kg	1.2	5.8
	3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)	EPA 8082	µg/kg	0.041	0.29
	3,3',4,4',5-Pentachlorobiphenyl (PCB 126)	EPA 8082	µg/kg	0.043	0.29
	3,3',4,4'-Tetrachlorobiphenyl (PCB 77)	EPA 8082	µg/kg	0.047	0.29
	3,4,4',5-Tetrachlorobiphenyl (PCB 81)	EPA 8082	µg/kg	0.05	0.29
	3,4,4'-Trichlorobiphenyl (PCB 37)	EPA 8082	µg/kg	0.052	0.29
	Decachlorobiphenyl (PCB 209)	EPA 8082	µg/kg	0.041	0.29
Pesticides/Herbicides					
	2,4'-DDD	EPA 8081A	µg/kg	0.13	0.58
	2,4'-DDE	EPA 8081A	µg/kg	0.16	0.58
	2,4'-DDT	EPA 8081A	µg/kg	0.058	0.58
	4,4'-DDD	EPA 8081A	µg/kg	0.11	0.58
	4,4'-DDE	EPA 8081A	µg/kg	0.11	0.58
	4,4'-DDT	EPA 8081A	µg/kg	0.17	0.58
Semivolatile Organics					
	Carbazole	EPA 8270C-SIM	µg/kg	2.3	2.8

Notes:
All analyses were performed by Columbia Analytical Services
MDL - method detection limit
MRL - method reporting limit

Table D4. Water analytical methods and associated method detection limits and method reporting limits.

Analyte Type - Lab	Analyte	Analytical Method	Units	MDL	MRL
Conventionals					
	Alkalinity	SM 2320B	mg/L	1 - 26.5	2 - 26.5
	Fluoride	EPA 300.0	mg/L	0.01 - 0.38	0.4 - 2
	Hardness as $CaCO_3$	SM 2340B	mg/L	0.4 - 2	0.4 - 2
	Organic carbon	SM 5310C	mg/L	0.02 - 24.5	0.05 - 24.5
	Sulfate	EPA 300.0	mg/L	0.01 - 0.2	0.2 - 4
	Total dissolved solids	SM 2540C	mg/L	5 - 205	5 - 205
Metals/Metalloids					
	Aluminum	EPA 6010B	µg/L	2.2	55.6
	Aluminum	EPA 6020	µg/L	0.3 - 242	2 - 242
	Antimony	EPA 6010B	µg/L	3.3	55.6
	Antimony	EPA 6020	µg/L	0.005 - 2.38	0.05 - 2.38
	Arsenic	EPA 6010B	µg/L	4.4	111
	Arsenic	EPA 6020	µg/L	0.1 - 3.3	0.5 - 10
	Barium	EPA 6010B	µg/L	0.4	5.6
	Barium	EPA 6020	µg/L	0.02 - 45.3	0.05 - 45.3
	Beryllium	EPA 6010B	µg/L	0.1	5.56
	Beryllium	EPA 6020	µg/L	0.006 - 0.12	0.02 - 0.4
	Cadmium	EPA 6010B	µg/L	0.3	5.6
	Cadmium	EPA 6020	µg/L	0.005 - 1.6	0.02 - 1.6
	Calcium	EPA 6010B	µg/L	6 - 9940	50 - 9940
	Chloride ion	EPA 300.0	mg/L	0.03 - 8.17	0.2 - 8.17
	Chromium	EPA 6010B	µg/L	0.7	5.6
	Chromium	EPA 6020	µg/L	0.02 - 8.62	0.2 - 8.62
	Cobalt	EPA 6010B	µg/L	0.4	11.1
	Cobalt	EPA 6020	µg/L	0.006 - 2.44	0.02 - 2.44
	Copper	EPA 6010B	µg/L	0.9	11.1
	Copper	EPA 6020	µg/L	0.02 - 13	0.1 - 13
	Iron	EPA 6010B	µg/L	0.8 - 222	20 - 444
	Lead	EPA 6010B	µg/L	4.4	55.6
	Lead	EPA 6020	µg/L	0.005 - 4.52	0.02 - 4.52
	Magnesium	EPA 6010B	µg/L	0.3 - 7240	20 - 7240
	Manganese	EPA 6010B	µg/L	0.2 - 0.22	5.56 - 5.6
	Manganese	EPA 6020	µg/L	0.006 - 11.1	0.05 - 11.1
	Mercury	EPA 1631E	ng/L	0.24 - 81.2	4 - 100
	Molybdenum	EPA 6010B	µg/L	0.7	11.1
	Molybdenum	EPA 6020	µg/L	0.007 - 2.21	0.05 - 2.21
	Nickel	EPA 6010B	µg/L	0.8	22.2
	Nickel	EPA 6020	µg/L	0.02 - 12.7	0.2 - 12.7
	Potassium	EPA 6010B	µg/L	40 - 2250	400 - 8890
	Selenium	EPA 6010B	µg/L	5.6	111
	Selenium	EPA 6020	µg/L	0.3 - 12	1 - 20
	Silicon	EPA 6010B	µg/L	4 - 414	200 - 444
	Silver	EPA 6010B	µg/L	0.8	11.1
	Silver	EPA 6020	µg/L	0.004 - 0.214	0.02 - 0.4
	Sodium	EPA 6010B	µg/L	20 - 13800	100 - 13800
	Thallium	EPA 6010B	µg/L	2.2	111
	Thallium	EPA 6020	µg/L	0.005 - 0.233	0.02 - 0.4
	Vanadium	EPA 6010B	µg/L	1.1	11.1
	Vanadium	EPA 6020	µg/L	0.03 - 2.26	0.2 - 4
	Zinc	EPA 6010B	µg/L	0.8	11.1
	Zinc	EPA 6020	µg/L	0.2 - 1250	0.5 - 1250

Notes:

All analyses were performed by Columbia Analytical Services

MDL - method detection limit

MRL - method reporting limit

Table D5. Statistical comparisons of total concentrations of copper, cadmium, lead, and zinc in sediment between site sediment samples and reference sediment samples.

	Cadmium		Copper		Lead		Zinc	
Site	GE	LALL	GE	LALL	GE	LALL	GE	LALL
DE	**	***	**	***	**	***	**	***
NP	*	**	*	**	*	**	*	**
LD	***	***	***	***	***	***	***	***
UMF	***	***	***	***	***	***	***	***
LMF	***	***	***	***	***	***	***	***

Statistical significance defined as $p \leq 0.01$ following a Bonferroni correction

$p \leq 0.01$ = *; $p \leq 0.005$ = **; $p \leq 0.001$ = ***

GE = Genelle Eddy
LALL = Lower Arrow Lake
DE = Deadman's Eddy
NP = North Port
LD = Little Dallas
UMF = Upper Marcus Flats
LMF = Lower Marcus Flats

Table D6. Statistical comparisons of dissolved concentrations of copper, cadmium, lead, and zinc between matrices of site sediment samples and reference sediment samples.

		Cadmium		Copper		Lead		Zinc	
Site	Matrix	GE	LALL	GE	LALL	GE	LALL	GE	LALL
DE	PW Suction	***		***	***	***	**	*	
	PW Peeper			***	***				***
	PW DGT	***	***	***	***			***	***
	SWI Suction	***	***	***	***	***	***	**	
	SWI Peeper							*	***
	SWI DGT	***	***	***	***			*	***
	OW	***	***	***	***	***	***	***	
NP	PW Suction	***		***	***				
	PW Peeper	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	PW DGT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	SWI Suction					***	***		
	SWI Peeper	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	SWI DGT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	OW			***	***	***	***	***	
LD	PW Suction	***		***	***			***	
	PW Peeper			***	***			**	***
	PW DGT	***	**	***	***			***	***
	SWI Suction			***	***	***	***	***	
	SWI Peeper				***				***
	SWI DGT			***	***				***
	OW			***	***	***	***	***	
UMF	PW Suction	***		***	***	***	***	**	
	PW Peeper			***	***			***	***
	PW DGT	***	***	***	***			***	***
	SWI Suction	***	***	***	***	***	***	**	
	SWI Peeper				*				***
	SWI DGT	***			***				***
	OW	**	**	***	***	***	***	***	
LMF	PW Suction		***	***	**	***	***	***	
	PW Peeper			**				***	***
	PW DGT	*				**		***	***
	SWI Suction			***	***	***	***	***	***
	SWI Peeper						**		***
	SWI DGT						**		***
	OW	*		***	***	***	***	***	***

Statistical significance defined as $p \leq 0.01$ following a bonferroni correction factor.

$p \leq 0.01$. = *; $p \leq 0.005$ = **; $p \leq 0.001$ = ***

GE = Genelle Eddy
LALL = Lower Arrow Lake
DE = Deadman's Eddy
NP = North Port
LD = Little Dallas
UMF = Upper Marcus Flats
LMF = Lower Marcus Flats

PW = Pore water
SWI = Sediment-Water Interface
OW = Overlying Water

Table D7. Statistical correlations of dissolved concentrations of copper, cadmium, lead, and zinc between sampling techniques (peepers, diffusive gradients in thin film [DGT], and active sampling through suction) in pore water (PW) and at the sediment-water interface (SWI).

	Cadmium		Copper		Lead		Zinc	
Sampling Techniques	PW	SWI	PW	SWI	PW	SWI	PW	SWI
Peepers and DGTs			*** ($p > 0.05$)	* ($p > 0.05$)			*** ($p > 0.05$)	* ($p < 0.05$)
Peepers and Suction	N/A		N/A	*** ($p < 0.05$)	N/A		N/A	
DGTs and Suction	N/A	* ($p < 0.05$)	N/A	* ($p > 0.05$)	N/A		N/A	

Statistically significant correlations are defined as $p \leq 0.05$ = *; $p \leq 0.01$ = **; $p \leq 0.001$ = ***. If correlations were observed between sampling techniques the Mann-Whitney U test was performed to assess whether concentrations of metal were statistically different; p values are presented in brackets.

N/A Not applicable because sampling technique was not performed in matrix

Table D8. Mean (± standard deviation) of general water quality parameters monitored at the University of Saskatchewan Aquatic Toxicology Research Facility during the duration of the study.

Treatment	pH (su)	Temperature (°C)	Conductivity (µS/cm)	DO (% Sat.)	Ammonia (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Overlying Water (OW)									
H2O	7.76 (± 0.176)	15.4 (± 0.94)	185 (± 18)	89.9 (± 5.5)	0.0517 (± 0.0354)	0.459 (± 0.33)	0.0482 (± 0.0607)	43.1 (± 3.14)	60.5 (± 3.7)
CTRL	7.76 (± 0.178)	15.4 (± 0.88)	189 (± 22.9)	89.7 (± 6.9)	0.037 (± 0.0274)	0.428 (± 0.357)	0.0422 (± 0.0302)	45.3 (± 5.46)	62.2 (± 4.4)
LALL	7.74 (± 0.185)	15.3 (± 0.82)	184 (± 16.8)	89.3 (± 5.4)	0.0404 (± 0.0221)	0.341 (± 0.228)	0.0286 (± 0.0177)	42.8 (± 4.28)	59.6 (± 4.03)
GE	7.69 (± 0.248)	15.4 (± 0.86)	187 (± 40.7)	88.8 (± 6.2)	0.0674 (± 0.174)	0.451 (± 0.325)	0.037 (± 0.0296)	42.8 (± 3.44)	60.3 (± 4.14)
DE	7.67 (± 0.293)	15.4 (± 0.99)	183 (± 17.8)	89.5 (± 5.4)	0.0393 (± 0.0307)	0.328 (± 0.21)	0.0388 (± 0.0259)	43.3 (± 3.23)	60.6 (± 3.84)
NP	7.73 (± 0.199)	15.4 (± 0.96)	184 (± 16.7)	88.8 (± 5.7)	0.0333 (± 0.0168)	0.556 (± 0.458)	0.0432 (± 0.0466)	42 (± 3.1)	60.1 (± 2.92)
LD	7.72 (± 0.199)	15.7 (± 0.77)	189 (± 98.2)	89 (± 5.5)	0.0377 (± 0.0248)	0.442 (± 0.361)	0.0393 (± 0.0283)	41.7 (± 3.71)	60 (± 4.03)
UMF	7.73 (± 0.211)	15.5 (± 0.85)	184 (± 17.3)	88.5 (± 6.1)	0.0398 (± 0.0252)	0.404 (± 0.357)	0.0359 (± 0.0333)	42.1 (± 2.98)	60.3 (± 3.99)
LMF	7.72 (± 0.191)	15.6 (± 0.88)	185 (± 17.8)	89.7 (± 5.5)	0.035 (± 0.0176)	0.428 (± 0.347)	0.0344 (± 0.032)	42.5 (± 2.93)	59.9 (± 5.37)
Sediment-Water Interface (SWI) Water									
H2O	ND	ND	ND	ND	ND	ND	ND	ND	ND
CTRL	7.58 (± 0.238)	16.6 (± 0.85)	192 (± 18.5)	88 (± 3.9)	0.0359 (± 0.0401)	0.425 (± 0.267)	0.0341 (± 0.0377)	46.4 (± 6.76)	60.6 (± 5.3)
LALL	7.46 (± 0.346)	16.5 (± 0.79)	188 (± 13.8)	88.9 (± 3.8)	0.0263 (± 0.00938)	0.333 (± 0.158)	0.0252 (± 0.0119)	41.8 (± 3.6)	59 (± 5.21)
GE	7.53 (± 0.165)	16.7 (± 0.83)	190 (± 15.3)	87.7 (± 4.1)	0.0278 (± 0.0129)	0.452 (± 0.32)	0.0292 (± 0.0176)	41.7 (± 4.41)	58.9 (± 2.84)
DE	7.5 (± 0.253)	16.5 (± 0.75)	188 (± 19.2)	88.6 (± 3.5)	0.0267 (± 0.00961)	0.369 (± 0.161)	0.0267 (± 0.02)	40.4 (± 4.77)	58.5 (± 3.19)
NP	7.53 (± 0.181)	16.7 (± 0.84)	188 (± 11.4)	86.8 (± 4)	0.0267 (± 0.0097)	0.362 (± 0.158)	0.0261 (± 0.0124)	42.4 (± 5.63)	60 (± 3.9)
LD	7.46 (± 0.256)	16.9 (± 0.83)	191 (± 17.1)	87.9 (± 4.7)	0.0248 (± 0.00863)	0.365 (± 0.164)	0.0255 (± 0.0122)	42.2 (± 3.57)	58.6 (± 3.7)
UMF	7.48 (± 0.239)	16.7 (± 0.81)	189 (± 16.7)	86.7 (± 4.5)	0.0267 (± 0.0135)	0.354 (± 0.173)	0.0244 (± 0.0109)	41.4 (± 2.9)	58.5 (± 4.32)
LMF	7.42 (± 0.287)	16.8 (± 0.78)	190 (± 19.2)	88.3 (± 3.5)	0.0257 (± 0.00917)	0.343 (± 0.118)	0.024 (± 0.0106)	41 (± 2.96)	59.6 (± 4.59)

Notes:

Test acceptability criteria:

Average water temperature should be 15 ± 1°C; with instantaneous measurements at 15 ± 3°C

Dissolved Oxygen (DO) should be ≥ 70%.

All other measurements should not vary by more than 50%.

CTRL - artificial substrate control

DE - substrates as collected above the water line from the gravel bar at DME

DME - Deadman's Eddy

GE - Genelle Eddy

H20 - water-only control

LALL - Lower Arrow Lakes

LD - Little Dalles

LMF - Lower Marcus Flats

NP - Northport

UMF - Upper Marcus Flats

Table D8. Mean (± standard deviation) of general water quality data during the study.

Treatment	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	DOC (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Potassium (mg/L)	Sulfate (mg/L)	Chloride (mg/L)
Overlying Water (OW)									
H2O	70.9 (± 44.1)	39.5 (± 4.45)	2.38 (± 3.83)	14.8 (± 17.7)	8.27 (± 0.791)	14.7 (± 1.76)	1.74 (± 0.193)	48.9 (± 6.18)	6.34 (± 0.599)
CTRL	65.1 (± 11.4)	43.1 (± 6.69)	1.97 (± 0.783)	12.8 (± 4.15)	8.05 (± 0.695)	14.1 (± 1.23)	1.67 (± 0.167)	48.4 (± 5.3)	6.21 (± 0.849)
LALL	61.9 (± 4.29)	38.7 (± 4.02)	2.72 (± 4.32)	11.3 (± 1.19)	8.16 (± 0.491)	14.2 (± 0.941)	1.71 (± 0.119)	48.9 (± 4.55)	6.31 (± 0.588)
GE	60.1 (± 10.9)	37.6 (± 6.95)	1.77 (± 0.446)	11 (± 2.1)	7.95 (± 1.43)	14.4 (± 1.14)	1.67 (± 0.3)	49.3 (± 4.63)	6.46 (± 0.9)
DE	75.5 (± 62.8)	38.8 (± 4.43)	2.28 (± 3.11)	16.8 (± 25.2)	8.16 (± 0.773)	14.9 (± 3.08)	1.72 (± 0.171)	48.9 (± 5.83)	6.4 (± 1.21)
NP	62.4 (± 3.56)	38.6 (± 3.31)	1.88 (± 0.525)	11.6 (± 0.781)	8.14 (± 0.57)	14.1 (± 0.978)	1.7 (± 0.137)	49.9 (± 5.87)	6.49 (± 0.678)
LD	64.4 (± 19.7)	39.8 (± 5.42)	2.24 (± 2.46)	12.2 (± 7.47)	8.22 (± 0.919)	14.5 (± 1.81)	1.72 (± 0.213)	49.2 (± 5.79)	6.33 (± 0.687)
UMF	61.3 (± 5.8)	38.6 (± 4.5)	1.82 (± 0.508)	11.2 (± 1.26)	8.09 (± 0.719)	14.2 (± 1.26)	1.7 (± 0.165)	48.4 (± 4.73)	6.22 (± 0.681)
LMF	76.4 (± 63.6)	39.5 (± 4.33)	2.22 (± 1.74)	16.5 (± 23.2)	8.56 (± 2.22)	15 (± 4.77)	1.79 (± 0.547)	48.8 (± 7.29)	6.38 (± 0.622)
Sediment-Water Interface (SWI) Water									
Pipette Suction Method									
CTRL	65.9 (± 10.4)	45.1 (± 7.01)	2.1 (± 1.29)	13.2 (± 3.53)	8.03 (± 0.757)	14.1 (± 1.22)	1.67 (± 0.168)	48.2 (± 4.6)	6.19 (± 0.736)
LALL	66.4 (± 40.1)	38.8 (± 4.16)	2.2 (± 1.32)	13.2 (± 15.8)	8.13 (± 0.607)	14.4 (± 1.36)	1.71 (± 0.167)	49.1 (± 4.2)	6.42 (± 1.24)
GE	61.5 (± 4.83)	39.3 (± 3.58)	1.95 (± 0.621)	11.1 (± 0.992)	8.2 (± 0.663)	14.4 (± 1.24)	1.74 (± 0.166)	49.1 (± 4.25)	6.38 (± 0.5)
DE	68.8 (± 28.6)	39.3 (± 4.99)	2.22 (± 2.36)	13.9 (± 9.9)	8.28 (± 1.3)	14.5 (± 1.88)	1.74 (± 0.217)	48.4 (± 5.94)	6.21 (± 0.764)
NP	61.2 (± 2.83)	39.4 (± 2.65)	2.11 (± 1.06)	11.2 (± 0.666)	8.07 (± 0.418)	14.3 (± 1.03)	1.7 (± 0.162)	49.2 (± 4.93)	6.27 (± 0.379)
LD	75.5 (± 93.2)	39.9 (± 5.74)	2.41 (± 2.17)	16.7 (± 36.7)	8.19 (± 0.85)	14.5 (± 1.94)	1.72 (± 0.196)	48.8 (± 5.82)	6.28 (± 0.788)
UMF	65.5 (± 25.6)	38.6 (± 4.44)	2.1 (± 0.851)	13 (± 10.1)	8.01 (± 0.605)	14.2 (± 1.25)	1.7 (± 0.164)	48.4 (± 5.09)	6.22 (± 0.753)
LMF	65.4 (± 25.9)	40 (± 4.35)	2.02 (± 0.662)	12.6 (± 9.33)	8.25 (± 0.863)	14.6 (± 2.25)	1.76 (± 0.32)	49.7 (± 5.34)	6.42 (± 0.687)
Peeper									
CTRL	ND	ND	ND	12.3 (± 1.95)	7.79 (± 0.913)	13.1 (± 1.51)	1.64 (± 0.38)	ND	ND
LALL	ND	ND	ND	10.3 (± 1.43)	7.89 (± 0.364)	13.3 (± 0.803)	1.89 (± 0.396)	ND	ND
GE	ND	ND	ND	9.98 (± 1.52)	7.77 (± 0.694)	13.1 (± 1.34)	1.93 (± 0.358)	ND	ND
DE	ND	ND	ND	11.1 (± 3.27)	7.84 (± 0.368)	13.1 (± 1.21)	1.7 (± 0.273)	ND	ND
NP	ND	ND	ND	ND	ND	ND	ND	ND	ND
LD	ND	ND	ND	10.7 (± 1.26)	8.08 (± 0.227)	13.6 (± 0.775)	1.8 (± 0.339)	ND	ND
UMF	ND	ND	ND	10.7 (± 1.13)	8 (± 0.315)	13.6 (± 0.874)	1.85 (± 0.365)	ND	ND
LMF	ND	ND	ND	10.3 (± 1.45)	7.82 (± 0.441)	13.2 (± 0.948)	1.86 (± 0.217)	ND	ND
Thin-Film Diffusive Gradient Probe									
CTRL	22.8 (± 6.28)	ND	ND	5.25 (± 1.7)	0.867 (± 0.273)	1.57 (± 0.633)	0.217 (± 0.0243)	ND	ND
LALL	18.2 (± 4.58)	ND	ND	3.5 (± 0.868)	1 (± 0.266)	1.93 (± 0.957)	0.23 (± 0.0448)	ND	ND
GE	16.7 (± 4.18)	ND	ND	3.28 (± 0.904)	0.901 (± 0.194)	1.72 (± 0.387)	0.221 (± 0.0254)	ND	ND
DE	56.3 (± 49.8)	ND	ND	13.5 (± 13.1)	4.41 (± 4.9)	2.25 (± 0.685)	0.252 (± 0.0457)	ND	ND
NP	ND	ND	ND	ND	ND	ND	ND	ND	ND
LD	32.2 (± 29.1)	ND	ND	7.58 (± 8.59)	1.98 (± 2.28)	1.66 (± 0.704)	0.226 (± 0.0291)	ND	ND
UMF	19 (± 5.39)	ND	ND	3.81 (± 0.984)	0.988 (± 0.328)	1.49 (± 0.52)	0.23 (± 0.0367)	ND	ND
LMF	21.9 (± 7.18)	ND	ND	4.33 (± 1.39)	1.16 (± 0.398)	1.17 (± 0.433)	0.224 (± 0.0237)	ND	ND

Treatment	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	DOC (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Potassium (mg/L)	Sulfate (mg/L)	Chloride (mg/L)
Porewater (PW) @ 1-cm									
Peeper									
CTRL	ND	ND	ND	21.5 (± 7.03)	7.96 (± 0.386)	13.4 (± 1.04)	3.02 (± 5.85)	ND	ND
LALL	ND	ND	ND	12.5 (± 1.61)	7 (± 1.15)	12.8 (± 0.83)	2.37 (± 0.473)	ND	ND
GE	ND	ND	ND	15.3 (± 3.64)	7.78 (± 0.536)	13.6 (± 0.52)	3.04 (± 0.795)	ND	ND
DE	ND	ND	ND	19 (± 6.11)	8.11 (± 0.953)	13 (± 0.859)	2.07 (± 0.214)	ND	ND
NP	ND	ND	ND	ND	ND	ND	ND	ND	ND
LD	ND	ND	ND	26.1 (± 9.22)	8.42 (± 0.787)	12.8 (± 1.19)	2.34 (± 0.52)	ND	ND
UMF	ND	ND	ND	23.9 (± 11.1)	7.84 (± 1.15)	13.1 (± 1.18)	2.97 (± 0.663)	ND	ND
LMF	ND	ND	ND	47.5 (± 10.2)	12.2 (± 1.46)	13.7 (± 0.996)	3.95 (± 0.421)	ND	ND
Thin-Film Diffusive Gradient Probe									
CTRL	19.3 (± 3.48)	ND	ND	4.67 (± 0.9)	0.638 (± 0.121)	1.63 (± 0.397)	0.217 (± 0.0239)	ND	ND
LALL	16.4 (± 4.49)	ND	ND	2.88 (± 0.675)	1.04 (± 0.349)	3.11 (± 2.23)	0.273 (± 0.113)	ND	ND
GE	15.2 (± 2.22)	ND	ND	2.87 (± 0.399)	0.876 (± 0.142)	1.71 (± 0.434)	0.217 (± 0.025)	ND	ND
DE	60.2 (± 62.2)	ND	ND	13.6 (± 15)	5.5 (± 6.67)	3.32 (± 1.59)	0.286 (± 0.0869)	ND	ND
NP	ND	ND	ND	ND	ND	ND	ND	ND	ND
LD	34.4 (± 34.1)	ND	ND	8.87 (± 11)	1.94 (± 2.17)	1.67 (± 0.862)	0.228 (± 0.0291)	ND	ND
UMF	16.7 (± 4.32)	ND	ND	3.51 (± 0.821)	0.79 (± 0.264)	1.9 (± 1.19)	0.239 (± 0.0369)	ND	ND
LMF	22.6 (± 2.71)	ND	ND	4.79 (± 0.566)	1.06 (± 0.237)	1.6 (± 0.548)	0.245 (± 0.0441)	ND	ND
Porewater (PW) @ 2.5-cm									
CTRL	104 (± 16.8)	ND	4.89 (± 2.86)	27.6 (± 5.16)	8.56 (± 1.02)	14.1 (± 0.77)	2.09 (± 0.34)	48.2 (± 4.19)	11 (± 25.2)
LALL	75.6 (± 8.94)	ND	6.9 (± 2.96)	21.4 (± 2.99)	5.39 (± 0.905)	13.1 (± 1.37)	4.17 (± 0.82)	45.8 (± 7.7)	6.86 (± 1.26)
GE	137 (± 48.5)	ND	16.4 (± 11)	40 (± 15.8)	9.28 (± 2.56)	17.3 (± 3.25)	7.96 (± 3.61)	28 (± 17.6)	7.64 (± 1.27)
DE	119 (± 32.6)	ND	7.35 (± 7.07)	36.1 (± 8.1)	7.97 (± 1.51)	14.9 (± 1.49)	3.22 (± 0.818)	57.9 (± 14.4)	8.54 (± 3.1)
NP	208 (± 42)	ND	8.96 (± 9.89)	62.3 (± 11.7)	12.8 (± 3.19)	14 (± 6.39)	3.79 (± 0.568)	81.9 (± 11.3)	8.05 (± 1.48)
LD	154 (± 31.8)	ND	9.52 (± 7.86)	44.3 (± 9.2)	10.6 (± 2.19)	11.7 (± 2.92)	2.98 (± 1.18)	60.4 (± 10.4)	7.63 (± 2.07)
UMF	180 (± 32)	ND	15.7 (± 9.7)	57.9 (± 10.5)	8.74 (± 1.75)	13.2 (± 1.97)	6.41 (± 1.3)	53.3 (± 8.21)	8.95 (± 11.2)
LMF	235 (± 41.8)	ND	25.4 (± 12.1)	70.2 (± 12.7)	14.6 (± 2.63)	15.1 (± 1.63)	6.12 (± 0.814)	50.1 (± 13.8)	8.34 (± 2.44)

Notes:

CTRL - artificial substrate control
DE - substrates as collected above the water line from the gravel bar at DME
DME - Deadman's Eddy
DOC - dissolved organic carbon
GE - Genelle Eddy
H20 - water-only control
LALL - Lower Arrow Lakes
LD - Little Dalles
LMF - Lower Marcus Flats
ND - non detect
NP - Northport
UMF - Upper Marcus Flats

Table D9. Required sample containers, preservation, and holding times for overlying water, sediment-water interface water, and pore water samples.

	Container [a]		Preservation	Holding Time	Proposed Minimum Laboratory Sample Size [b]
	Type	Size			
Conventional Parameters					
Alkalinity as $CaCO_3$	HDPE	1000 mL	4±2ºC	14 days	25 mL
Dissolved organic carbon	HDPE	250 mL	H_2SO_4 to pH <2; 4±2ºC	28 days	25 mL
Hardness as $CaCO_3$	HDPE	with metals	1 mL of 1:1 HNO_3; 4±2ºC	6 months	with metals [e]
Total dissolved solids	HDPE	with alkalinity	4±2ºC	7 days	200 mL
Total suspended solids	HDPE	with alkalinity	4±2ºC	7 days	200 mL
Major Ions					
Calcium, magnesium, potassium, sodium	HDPE	with metals	1 mL of 1:1 HNO_3; 4±2ºC	6 months	with metals [e]
Chloride, fluoride, sulfate	HDPE	250 mL	4±2ºC	28 days	5 mL
Nutrients					
Ammonia	HPDE	250 mL	H_2SO_4 to pH <2; 4±2ºC	28 days	5 mL
Nitrate + nitrite	HDPE	250 mL	H_2SO_4 to pH <2; 4±2ºC	28 days	5 mL
Common metals and metalloids [c]	HDPE	250 mL	1 mL of 1:1 HNO_3; 4±2ºC	6 months	15 to 20 mL
Mercury [d]	FP or G with FP-lined lids	500 mL	BrCl in lab within 28 days of collection; 4±2ºC	90 days	100 mL

Notes:
BrCl = bromine chloride
FP = fluoropolymer
G = glass
HDPE = high density polyethylene bottle
H_2SO_4 = sulfuric acid
HNO_3 = nitric acid
[a] Sample container sizes may be modified to meet laboratory requirements
[b] Extra sample volume will be collected at a frequency of 5 percent of samples to accommodate requirements for laboratory quality control samples.
[c] Surface water samples will be collected and analyzed for dissolved metals and metalloids. A total of 1 L of water will be collected for the common metals/metalloids analyses (dissolved), and 500 mL will be collected for analysis of mercury.
[d] Due to volume limitations mercury analyses will not be performed on porewater samples.
[e] These analyses will be conducted from the same sample collected for metals analysis; therefore, no additional volume is required.

APPENDIX D1

Estimating summary statistics for datasets that include below detection limit values

In the analysis of water quality data that accompanies the 2010 chronic sturgeon sediment exposure, reported concentrations as measured on a parts per billion (ppb) basis were frequently below analytical detection limits (BDLs). As a result, the true concentrations for chemicals of potential concern (e.g., dissolved metals) lay somewhere between zero and the analytical method detection limit (MDL) or method reporting limit (MRL). To enable the use of this data (i.e., censored data) in evaluating summary statistics such as arithmetic- and geometric means, and standard deviations, maximum likelihood estimation (MLE) procedures were used. Procedures such as MLE provide better estimates of summary statistics for censored data (e.g., BDLs) than simple "blind" calculations that treat BDLs as detected measurements or 'fabricating' values with the use of archaic substitution (e.g., one-half the value of the detection limit) methods (Helsel 1990). The following appendix outlines the application and subsequent evaluation (via Monte Carlo methodology) of several methods based on MLE or alternative procedures applied to data containing BDLs. Four methods were tested in this comparison and include the "censored MLE" (CENMLE) and "regression on order statistics" (ROS) procedures built in to the R-statistical package (Helsel 2005), the MLE procedure built in to the Biotic Ligand Model (BLM; HydroQual 2009), and a "blind" calculation method that treats the BDL values as regular measurements.

To evaluate these methods, a Monte Carlo procedure was used to generate a sample dataset from a known distribution. Estimates of the geometric mean and standard deviation from each of the methods could then be compared to the known answer to evaluate the accuracy and

precision of each method. Sample datasets generated by Monte Carlo were intended to resemble the types of metal concentration data encountered in the study. Values for geometric mean, standard deviation, number of data points, and fraction of data that were BDL were all chosen to resemble the actual metal data. For a given distribution with specified geometric mean and standard deviation, individual data points were randomly generated, some noise representing plus or minus 10 percent of the value was introduced to represent analytical variability, and a detection limit was then chosen so that a specified fraction of the available data were BDL. Different values of the fraction BDL were used ranging from 20 percent to 80 percent of the total number of data to test these methods over a range of conditions representative of metal concentration data in the 2010 sturgeon database. An example dataset is shown in Figure D1.1. For these example data, there are 10 data points and 70 percent of them are BDL. The "true" lognormal distribution used to generate the data is shown as the black diagonal line, and represents a dataset with a geometric mean of 0.017, and standard deviation of 0.75.

For this example, the 10 data points were then supplied to four different estimation procedures to evaluate how well these methods could estimate summary statistics. Values that were BDL were replaced by the detection limit (as shown in Figure D1.1).

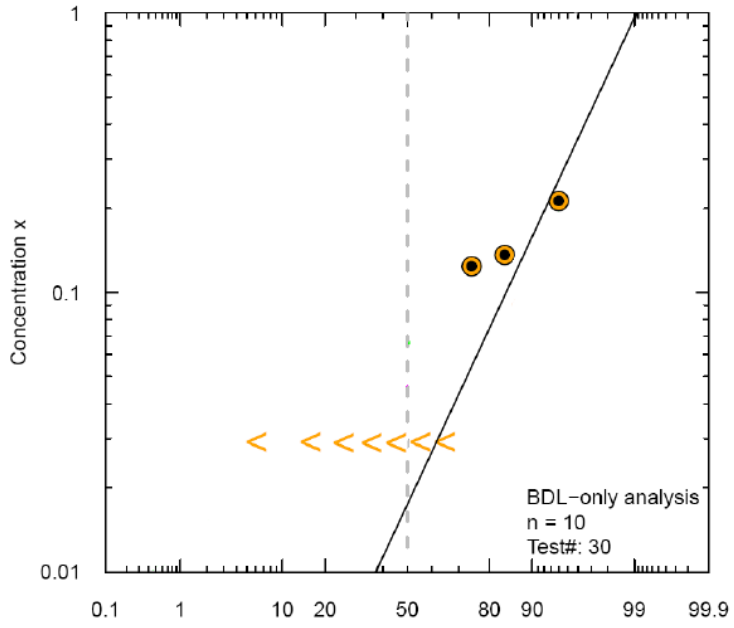


Figure D1.1. An example test dataset generated for a Monte Carlo evaluation of numerical procedures to estimate statistical distributions for data sets that include values below an analytical detection limit.

Note: The true distribution is a lognormal distribution with a geometric mean of 0.017, and standard deviation of 0.75 and is shown with the solid black line. Ten random sample points were generated from this distribution. To each data some random noise was added. A detection limit was selected and any points with values below the detection limit were replaced with the detection limit. Observations that remain above the detection limit are shown as circles and those below the detection limit are shown as less-than (i.e. "< ") symbols plotted at the value of the detection limit. The geometric mean, standard deviation, number of points, and proportion of data below the detection limit were all selected to be similar to actual metal datasets produced as part of the chronic sturgeon study.

The MLE procedures are based on optimization of a likelihood function (Shumway et al. 2002). For a given dataset with *n* observations, the likelihood function is based on the following equations:

$$L = \prod_{i=1}^{n} P(x_i)^{1-\delta_i} \times C(x_i)^{\delta_i} \qquad \text{(Equation D1.1)}$$

Where, for the likelihood function *L* across *n* observations *P(x)* is the probability density function for a normal distribution used for non-BDL values of *x*, and *C(x)* is the cumulative density function used for BDL values of *x*. For a given mean (μ) and standard deviation (σ), the probability density function is:

$$P(x) = \frac{exp\left[-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2\right]}{\sigma\sqrt{2\pi}} \qquad \text{(Equation D1.2)}$$

For the same distribution, a cumulative density function is defined as:

$$C(x) = \frac{1}{2}\left[1 + \text{erf}\left(\frac{x-\mu}{\sqrt{2\sigma^2}}\right)\right] \qquad \text{(Equation D1.3)}$$

Where erf is the Gauss error function (Andrews 1997). For detected observations, the censored flag δ is 0, so only the term for P(x) is used in the likelihood function, and the C(x) term will drop out. For BDL observations, the censored flag δ is 1 and the P(x) term will drop out. The goal of the MLE procedure is to find a mean (μ) and standard deviation (σ) that maximizes "*L*" (Equation E-1) for a given dataset, that includes both detected and BDL observations. For a

log-normally distributed dataset, a geometric mean can be found by applying the MLE to log-transformed values of *x*. The "blind" calculation was included to allow comparison of MLE methods that consider BDL values against a simple alternative to demonstrate the benefit of incorporating these methods into the overall analysis. As shown in the pink line in Figure D1.2 if the BDL data are treated the same as other measurements, calculation of the geometric mean tends to produce a value that is higher than the true value, and the estimate of the standard deviation is lower than the true value. For this example, the resulting "blind" estimate of the geometric mean is 0.047, compared to an actual value of 0.017; while the estimate of the standard deviation is 0.36, compared to an actual value of 0.75.

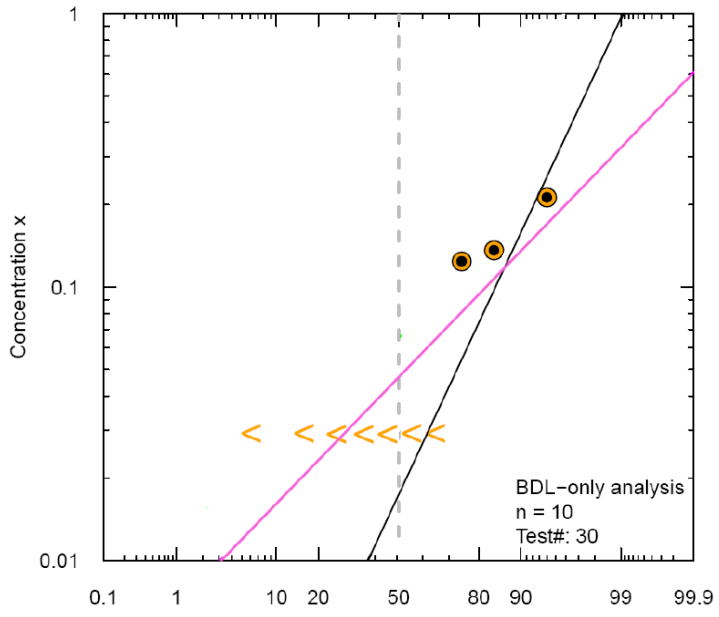


Figure D1.2. Example "blind" calculation permitting the comparison of MLE methods that consider BDL values against a simple alternative.

Note: If below detection limit values shown in Figure D1.1 are treated as actual measured values, the resulting geometric mean and standard deviation are biased from the true values. This "blind" characterization of the distribution (shown as the solid pink line) tends to over-estimate the geometric mean (estimate 0.047, actual value 0.017) and underestimate the standard deviation (estimate 0.36, actual value 0.75) relative to the true distribution (the solid black line).

Consideration of BDL values using a MLE procedure results in estimated summary statistics that are much closer to the true values (Figure D1.3) (estimated geometric mean of 0.011 and standard deviation of 0.48). This type of comparison was repeated with a Monte Carlo procedure to generate 4000 independent datasets. Results for all 4000 comparisons of the geometric mean are shown in Figure D1.4. For each estimate, the ratio of the estimated value of the geometric mean to the true value is shown as a histogram. Results from the CENMLE procedure produce a histogram that is nearly centered around a ratio of 1.0, see Figure D1.4 Panel A. Results from the ROS procedure (Helsel 2005) are shown in Figure D1.4 Panel B for comparison. A second MLE method incorporated in the BLM (HydroQual 2009), differs from the CENMLE procedure in that it only assumes that the fraction of the data that are BDL are normally distributed. This method was included in this comparison since the general use of the BLM in the analysis of sturgeon and other metals effects data make this built-in procedure an attractive alternative in subsequent data analyses. For these data, the BLM based MLE produced results that were comparable to CENMLE (Figure D1.4 Panel C). The ROS procedure typically produced estimates that were somewhat more variable compared to the true value than either of the MLE procedures (i.e., the histogram in Panel B is broader, indicating that there were a higher proportion of ROS estimates with larger deviations from the true value). The blind calculation that treats BDL data the same as detected observations shows a consistent tendency to overestimate the geometric mean (Figure D1.4 Panel D).

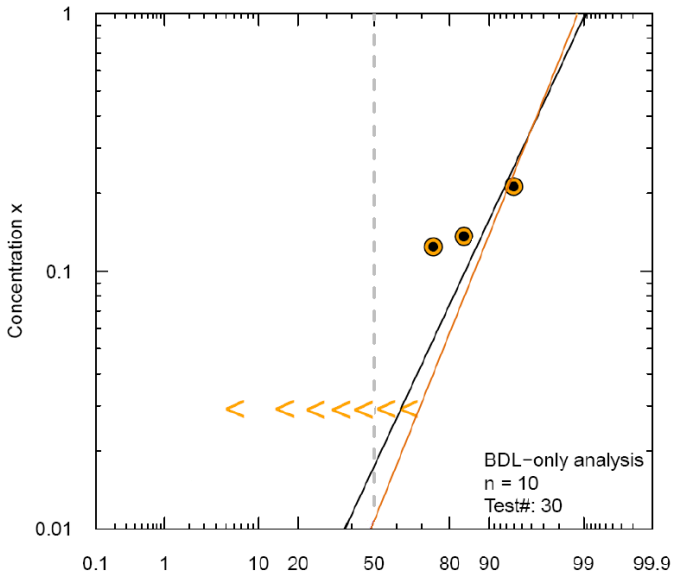


Figure D1.3. Example calculation incorporating BDL values using MLE methods.

Note: Summary statistics for datasets that include values below analytical detection limits can be estimated using MLE techniques. An estimate of the distribution using MLE is shown for the sample data described in Figure D1.1 (brown line). The MLE estimates of the geometric mean (0.011) and standard deviation (0.48) are closer to the true distribution (shown in black) than a blind calculation that does not consider BDL values (shown in Figure D1.2).

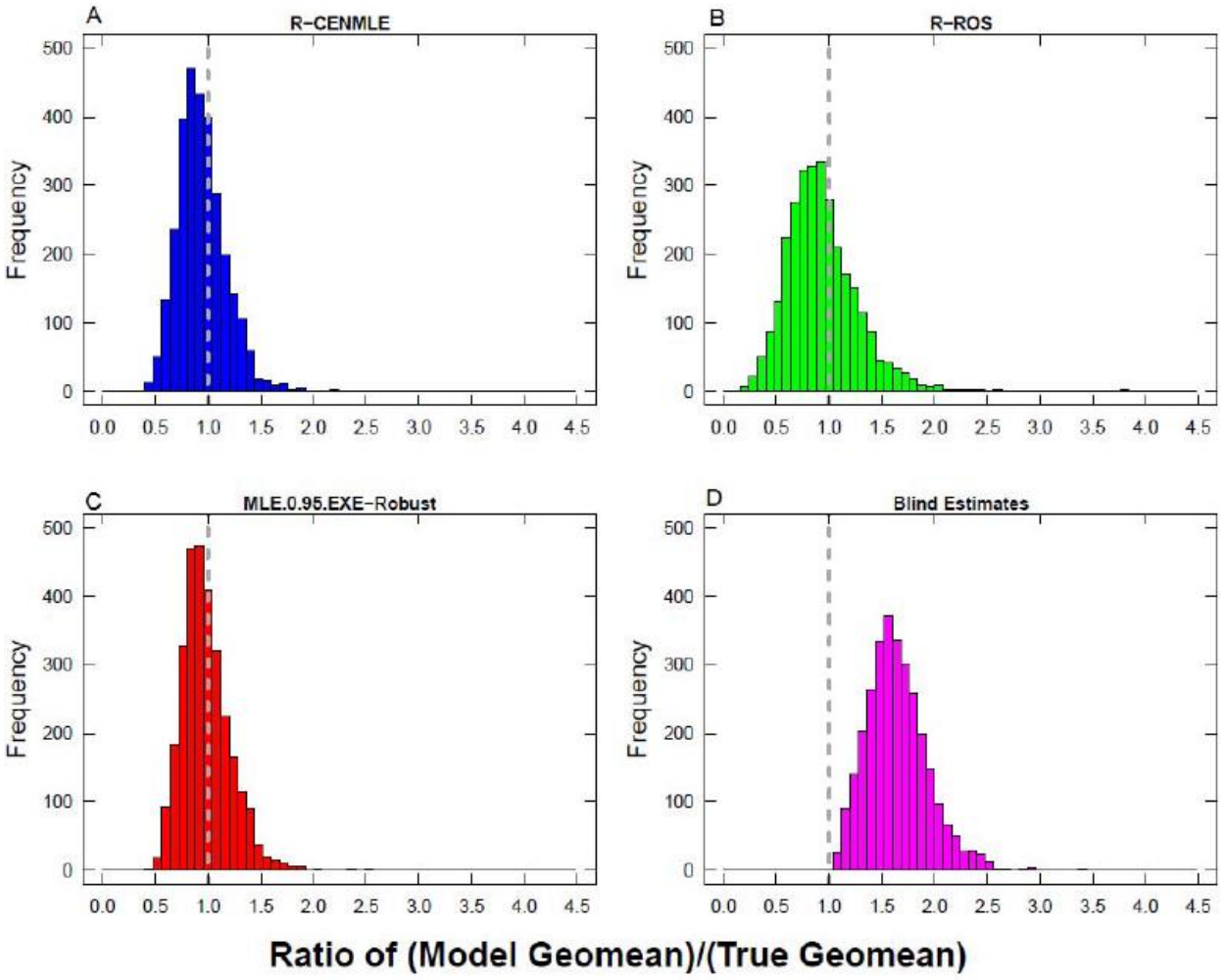


Figure D1.4. Frequency histograms for 4000 estimates of the geometric mean from 4000 different synthetic datasets generated as part of the Monte Carlo evaluation.

Note: Results for four estimation methods are shown, including the CENMLE and ROS procedures in the R-statistical package (Panels A and B; Helsel 2005), the MLE procedure built into the BLM (Panel C) and a blind calculation that treats BDL as normal measurements (Panel D). Estimated values are shown as a ratio to the actual geomean. For this comparison 40 percent of the synthetic data were replaced by a detection limit value.

Similar conclusions are reached from comparisons of the estimates of the standard deviation (see Figure D1.5). Both MLE methods and ROS produce histograms centered around a value of 1, indicating that there is no systematic bias in these methods. However, estimates from the ROS method tend to deviate from the true values more frequently (and hence a broader

histogram in Figure D1.5 Panel B). The blind calculation shows a systematic bias with estimates of standard deviation consistently lower than the true values.

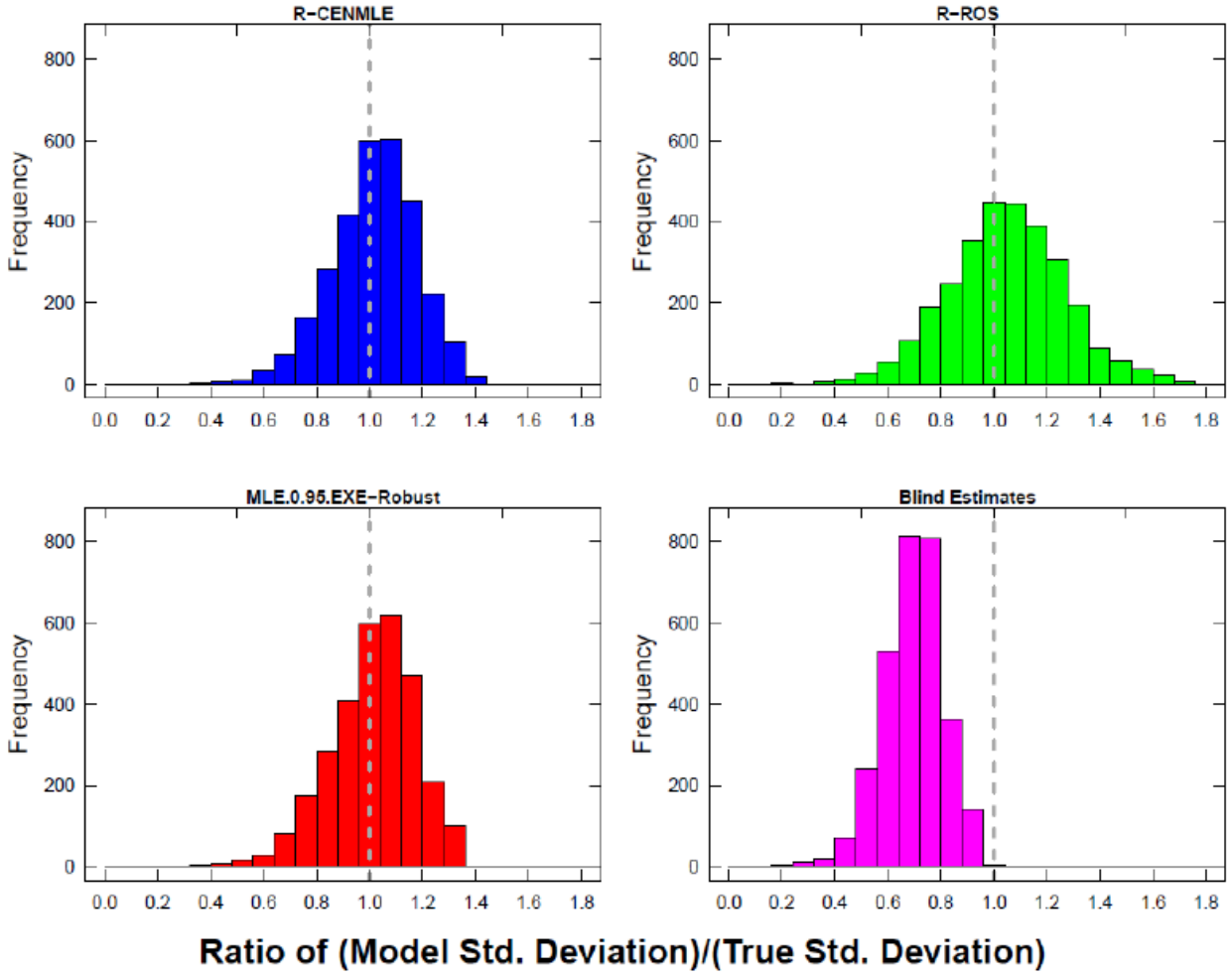


Figure D1.5. Frequency histograms for 4000 estimates of the standard deviation from 4000 different synthetic datasets generated as part of the Monte Carlo evaluation.

Note: Results for four estimation methods are shown, including the CENMLE and ROS procedures in the R-statistical package (Panels A and B; Helsel 2005), the MLE procedure built into the BLM (Panel C) and a blind calculation that treats BDL as normal measurements (Panel D). Estimated values are shown as a ratio to the actual standard deviation. For this comparison 40 percent of the synthetic data were replaced by a detection limit value.

The Monte Carlo evaluation shown in Figures D1.4 and D1.5 were repeated with percentages of data assigned as BDL ranging from 20 to 80 percent and number of data points from 10 to 30. Results from all cases were comparable to the 40 percent BDL dataset show in Figures D1.4 and D1.5. The MLE methods consistently performed marginally better than ROS and considerably better than a blind estimate that did not consider BDLs. As a result and given that the R-statistical software package was used extensively in the evaluation and graphing of 2010 sturgeon data, the CENMLE procedure (an internal function in the R software) was chosen for the analysis of metal concentration data. Application of the CENMLE procedure was limited to datasets which had BDL values ≤ 80 percent of the total number of observations, as the procedure has been noted by others to be unreliable when more than 80 percent of the data correspond to BDL values (Helsel 2005). For datasets with greater than 80 percent BDLs, an upper bound to the geometric mean was estimated by using the detection limit values in the averaging procedure. Monte Carlo analysis shows that this result is always greater than the actual geometric mean, and values estimated this way are therefore shown with a less than symbol (i.e., "<") to indicate that the result is known to be lower than the estimated value.

The results from this comparison are consistent with recommendations by Helsel (2005, p.78) that either MLE or ROS would be appropriate for small datasets with percent censored values that are 80 percent or fewer of the total data. The demonstrated performance of these methods in this Monte Carlo analysis is particularly relevant to the 2010 sturgeon dataset, since parameters such as number of data points, geometric mean, and standard deviation were all chosen to be representative of metal concentration data from this study. The performance summarized in Figures D1.4 and D1.5 is therefore representative of the expected performance of

the MLE procedure used in the analysis of the metals concentration data in the 2010 sturgeon database. For datasets where BDL values represent more than 80 percent of the total data available, MLE and ROS procedures are less reliable, and in these cases a "blind" estimate of the geometric mean can be used, with the acknowledgment that it is a conservative estimate shown to be biased to values greater than the actual value (as in Figure D1.4, Panel D).

The MLE procedure was also used to develop box-and-whisker plots for data that included BDL values. An example of this application is shown in Figure D1.6. The box in the box and whisker plot is defined such that the upper edge of the box corresponds to the 75th percentile of the original data, and the bottom edge of the box corresponds to the 25th percentile, and the whiskers extend to maximum and minimum values exclusive of extreme values. Application of the box and whisker to the true distribution from the aforementioned example is shown as the gray box in Figure D1.6 Panel A. Vertical lines show where the true distribution (black line) intersects the 25th and 75th percentile. At these intersections, horizontal lines read across to the lower and upper edge of the box. The geometric mean for this distribution is equivalent to the median (50th percentile), since it is log-normally distributed, and this value is shown as a horizontal line in the middle of the box.

If BDL values are not considered, and are treated the same as detected values, the result will over-estimate the geometric mean, and under-estimate the standard deviation as is shown in the pink box in Figure D1.6 Panel B. These graphical discrepancies are consistent with the numerical discrepancies seen in Figures D1.4 and D1.5.

If MLE methods are used to consider BDL values, a much better estimate of the true distribution results such that the red box in Figure D1.6 Panel C is nearly identical to the gray box that results from the true distribution. It is important to note, however, that the resulting box and whisker plot that results from the MLE analysis appears to produce a geometric mean that is lower than all of the observed data (i.e., measured values and detection limits for samples that are BDL). This apparent discrepancy results from the fact that a high proportion of the observed data are actually BDL values plotted at the detection limit. The real values that correspond to these BDL values are, by definition, lower than the detection. The MLE procedure considers this fact, and produces an estimate of the geometric mean accordingly. Similar box and whisker plots that correspond to metal concentrations in the chronic sturgeon exposure chambers frequently exhibit similar behavior, and the comparably low geometric means evident in those figures are likewise an understandable and expected consequence that results from a high proportion of BDL values in the metals datasets.

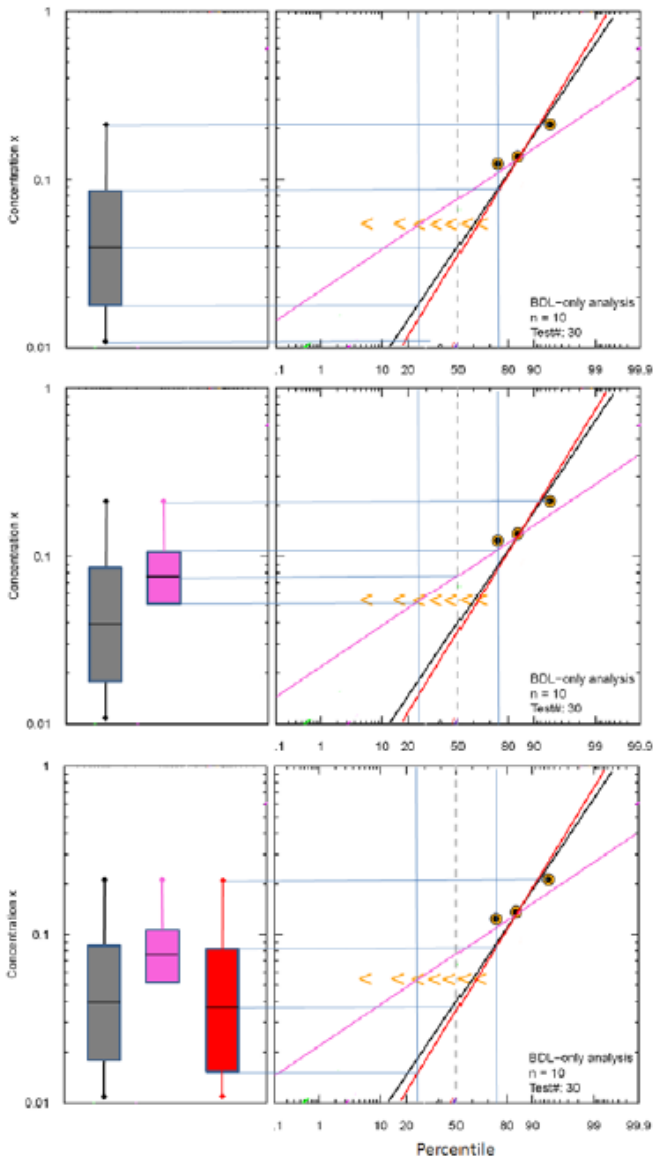


Figure D1.6. Box-plots of MLE results for datasets that include BDL values

Note: Data are the same in Figure D1.1 and are drawn at random from a distribution (black line). For each box plot, the upper edge of the box represents the 75th percentile, the lower edge the 25th percentile, and the horizontal line in the middle of the figure represents the geometric mean. Whiskers above and below the box extend to min and max values. In Panel A the box plot (gray) is developed from the true distribution. In Panel B, the measurements are used to develop the box plot (pink) without considering that some values are below detection limit and show the typical overestimation of the mean, and underestimation of the standard deviation. In Panel C, the summary statistics for the box plot (red) were derived from the MLE estimate of the distribution, as characterized by the geometric mean and standard deviation.

SUMMARY

- Metals concentration datasets in the chronic sturgeon exposures frequently include values that are below BDLs.

- A Monte Carlo analysis showed that ignoring the presence of BDLs resulted in systematic errors in estimates of the mean and standard deviation and should be avoided.

- Consideration of BDLs using either MLE or ROS produced unbiased estimates of the mean and standard deviation, and of these two methods the MLE procedure produced an accurate result.

- The MLE procedure, and specifically the CENMLE procedure in the R-statistical software package, was chosen for the analysis of metal concentrations in chronic sturgeon exposures to produce summary statistics, and summary graphics (e.g., box and whisker plots).

- Application of the MLE was limited to datasets which had 80 percent or fewer observations flagged as BDLs. For datasets with more than 80 percent BDLs, an upper bound on the geometric mean was calculated using the reported detection limit values.

APPENDIX D2

Validation Assessment of Analytical Data for Upper Columbia River Sediment Toxicity Tests

Environmental Standards Inc. (ESI; Valley Forge, PA, USA)) performed an independent quality assurance and data validation review of the white sturgeon sediment toxicity data produced by Columbia Analytical Services (CAS) of Kelso, Washington (WA). The review was performed following EPA guidance documents:

"Guidance for Labeling Externally Validated Laboratory Analytical Data for Superfund Use" (EPA-540-R08-008) (USEPA 2009)

"U.S. EPA Contract Laboratory Program National Functional Guidelines for Organic Data Review" (EPA/540/R-99/008) (USEPA 1999)

"U.S. EPA Contract Laboratory Program National Functional Guidelines for Inorganic Data Review" (EPA/540/R-04-004) (USEPA 2004)

Data were examined to determine usability of the analytical results and compliance relative to requirements specified above and the analytical methods. In addition, deliverables were evaluated for completeness and accuracy. Qualifier codes have been placed next to results on the data tables to enable the data user to quickly assess the qualitative and/or quantitative reliability of any result based on the criteria evaluated. EPA's Quality Assurance/Quality Control (QA/QC) chemist reviewed the draft data and data validation reports. Issues were resolved and EPA approved the data for public. The following sections summarize results of the validation. It should be noted that general in-house water quality parameters monitored (e.g., temperature, pH, DO, conductivity, and inorganic nitrogen) during the tests were not validated by ESI. These routine water quality parameters were monitored to ensure that toxicity tests were within design constraints at the time of testing.

Overall data quality

Most analytical data were useable, with qualifications presented in data validation reports and included in the project database. Only useable data were included in this report, although all rejected data are in the project database. Data qualifiers were assigned to data by the laboratory and validators to signify when data were out of calibration range (i.e., below or above levels of quantification), where contaminated blanks compromised data interpretability, or if matrix spikes, internal standards, or other quality control metrics were exceeded. Tables summarizing the number of samples with each type of data qualifier by analyte and information regarding how data qualifiers were used, along with full data validation reports, are available upon request from the corresponding author.

Of the 8,482 analytical data points collected during this Study, a total of 1,014 data points (<12 percent) were rejected as follows:

- Results for all organochlorine pesticide compounds, PCB congeners, and PAH compounds in 910 sediment data points were qualified as unusable due to exceeded sample preservation temperatures.
- Results for molybdenum 20 data points were qualified as unusable due to very low reporting limit (RL) standard recoveries.
- Results for calcium, iron, and/or molybdenum in 84 aqueous samples were qualified as unusable due to very low RL standard recoveries.

Sample transport and holding times

Validity of analytical data was evaluated with regard to sample preservation conditions and sample holding times from the date of collection to the date of analysis. Aqueous and sediment samples were to be preserved at 4 ± 2°C. Most samples received by the laboratory were within the required preservation temperature range. In cases where the sample receipt temperature was outside of the study-required range, and the associated analytical method did not specify preservation criteria, data were not qualified. Results for PCB congeners and PAH compounds in

several sediment samples were qualified as estimated due to receipt temperatures above the method-specified range. In a limited number of instances, aqueous samples submitted for metals analyses could not be analyzed for pH by the laboratory due to limited sample volume. Aqueous samples for metals analysis were to be preserved to a pH < 2 su. As a result, there was insufficient information for ESI to verify that the affected samples were properly preserved; and data were not qualified due to this issue. No transport holding times were exceeded.

Equipment Rinse Blanks

Results for calcium, iron, magnesium, sodium, aluminum, barium, copper, lead, manganese, molybdenum, nickel, zinc, cadmium, chromium, cobalt, silver, and/or antimony in several sediment samples were qualified as "not-detected" due to the presence of these analytes at similar concentrations in the associated rinse blanks.

Results for aluminum, antimony, barium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, potassium, silicon, silver, sodium, vanadium, zinc, mercury, chloride, fluoride, dissolved organic carbon, and/or total dissolved solids in several aqueous samples were qualified as "not-detected" due to the presence of these analytes at similar concentrations in the associated rinse blanks.

Laboratory Holding Times

Results for organochlorine pesticide compounds, PCB congeners, AVS/SEM, TOC, pH, and/or grain size in several sediment samples were qualified as estimated due to holding time exceedance.

Inorganics

All inorganic analyses were conducted by CAS in Kelso, WA. Overall, the data reviewed are usable with the qualifications detailed in the validation reports and database.

Calibration

Results for thallium, selenium, beryllium, cobalt, iron, molybdenum, magnesium, calcium, and/or copper in several sediment samples were qualified as estimated due to out-of-criteria RL standard recoveries.

Results for thallium, sodium, beryllium, calcium, iron, copper, molybdenum, cobalt, magnesium, nickel, selenium, potassium, and/or manganese in several aqueous samples were qualified as estimated due to out-of-criteria RL standard recoveries.

Blanks

Results for iron, molybdenum, magnesium, selenium, thallium, calcium, arsenic, chromium, and/or nickel in several sediment samples were qualified as "not-detected" due to the presence of these analytes at similar concentrations in the associated laboratory blanks.

Results for beryllium, cobalt, iron, magnesium, manganese, molybdenum, potassium, silicon, silver, thallium, zinc, arsenic, and/or selenium in several aqueous samples were qualified as "not-detected" due to the presence of these analytes at similar concentrations in the associated laboratory blanks. Results for sodium, calcium, chromium, copper, iron, potassium, molybdenum, thallium, and/or magnesium in several sediment samples were qualified as estimated due to significant negative instrument bias in the associated laboratory blanks.

Results for chromium, copper, potassium, calcium, iron, silicon, molybdenum, aluminum, magnesium, and/or sodium in several aqueous samples were qualified as estimated due to significant negative instrument bias in the associated laboratory blanks.

Matrix Spikes

Results for magnesium, potassium, aluminum, lead, and/or barium in several sediment samples were qualified as estimated due to out-of-criteria matrix spike recoveries.

Laboratory Control Samples

Results for iron and total dissolved solids (TDS) in several aqueous samples were qualified as estimated due to out-of-criteria laboratory control sample recoveries.

Laboratory and Field Duplicates

Results for arsenic, copper, iron, barium, aluminum, and/or grain size in several sediment samples were qualified as estimated due to laboratory duplicate imprecision.

Results for chromium, lead, zinc, and/or TDS in several aqueous samples were qualified as estimated due to laboratory duplicate imprecision.

Results for lead, thallium, mercury, barium, copper, nickel, zinc, cadmium, aluminum, antimony, chromium, manganese, vanadium, alkalinity, TDS, and/or dissolved organic carbon (DOC) in several aqueous samples were qualified as estimated due to field duplicate imprecision.

Interference Check Samples

Results for beryllium, magnesium, and/or nickel in several sediment samples were qualified as estimated due to inductively-coupled plasma (ICP) interference.

Serial Dilutions

Results for calcium, chromium, lead, magnesium, and/or nickel in several sediment samples were qualified as estimated due to serial dilution imprecision. Results for sodium, copper, aluminum, molybdenum, antimony, manganese, iron, zinc, magnesium, chromium, arsenic, barium, cadmium, cobalt, lead, nickel, vanadium, and/or calcium in several aqueous samples were qualified as estimated due to serial dilution imprecision.

Polychlorinated biphenyl congeners

Analysis of samples for PCB congeners was conducted by CAS in Kelso, WA. Overall, data are usable with the qualifications presented in the validation reports and database.

Calibration

Results for PCB-149 in several sediment samples were qualified as estimated due to a calibration issue.

Blanks

An evaluation of laboratory blanks associated with PCB congener analyses revealed no contamination issues.

Co-elution

An evaluation of chromatograms associated with PCB congener analyses showed acceptable peak resolution and no co-elution issues.

Surrogate Recovery

All PCB congener surrogate recoveries were within acceptance limits.

Matrix Spikes

All PCB congener matrix spike results were within acceptance limits.

Laboratory Control Samples

All PCB congener laboratory control sample results were within acceptance limits.

Organochlorine pesticide compounds

Analysis of samples for organochlorine pesticide compounds was conducted by CAS in Kelso, WA. Overall, data are usable with qualifications presented in the validation reports and database.

Calibration

Instrument stability was demonstrated for organochlorine pesticide analyses and all initial and continuing calibration results were within acceptance limits.

Blanks

An evaluation of laboratory blanks associated with organochlorine pesticide analyses revealed no contamination issues.

Surrogates

All organochlorine pesticide surrogate recoveries were within acceptance limits.

Matrix Spikes

All organochlorine pesticide matrix spike results were within acceptance limits.

Laboratory Control Samples and Standard Reference Material

Results for 4,4'-DDD and 4,4'-DDE in two sediment samples were qualified as estimated due to low standard reference material recoveries.

Polyaromatic hydrocarbon compounds

Analysis of samples for PAH compounds was conducted by CAS in Kelso, WA. Overall, the data are usable with the qualifications presented in the validation reports and database.

Calibration

Instrument stability was demonstrated for PAH analyses and all initial and continuing calibration results were within acceptance limits.

Blanks

Results for naphthalene in several sediment samples were qualified as ”not-detected” due to its presence at a similar concentration in an associated laboratory blank.

Surrogates

All PAH surrogate recoveries were within acceptance limits.

Matrix Spikes

All PAH matrix spike results were within acceptance limits.

Laboratory Control Samples

All PAH laboratory control sample results were within acceptance limits

APPENDIX D3

Sediment data distribution plots for white sturgeon toxicity testing

Inorganics

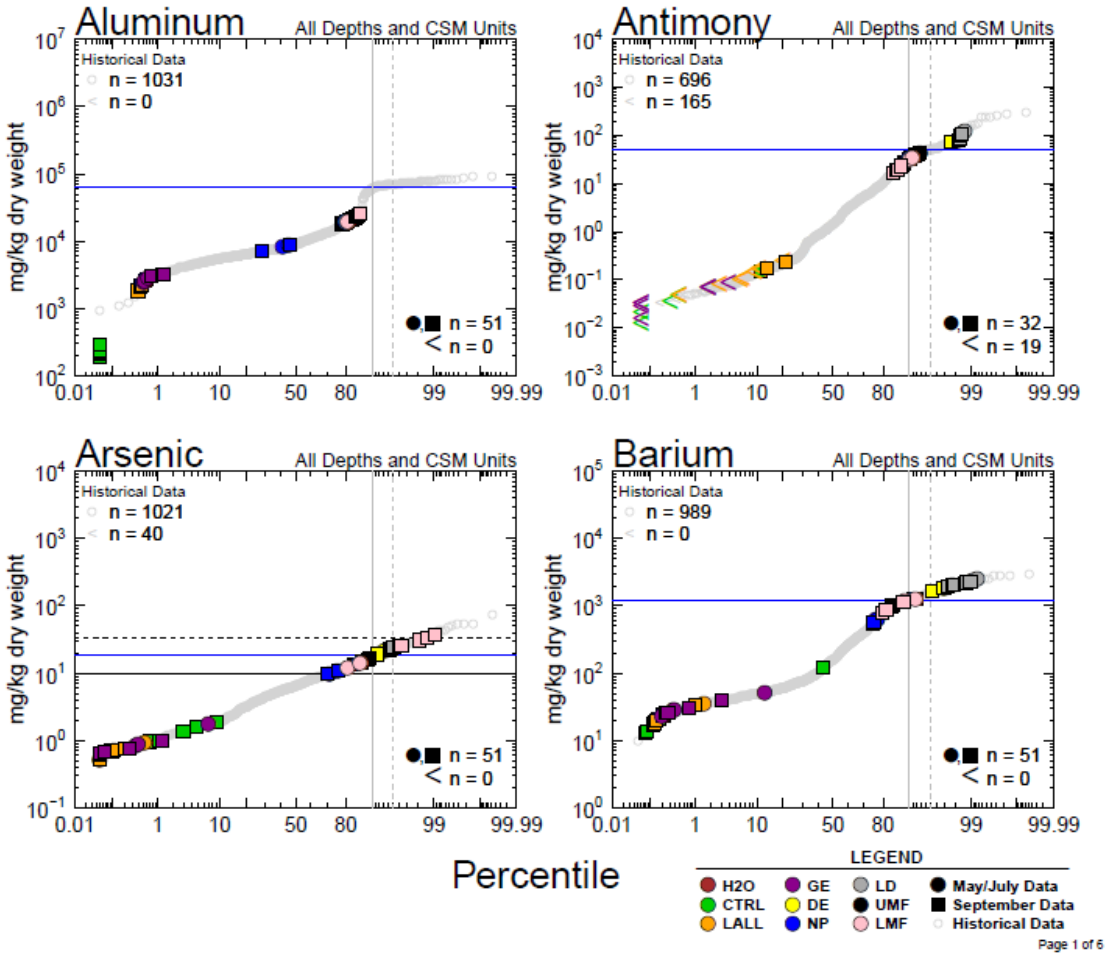


Figure D3.1. Bulk metal concentration in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Metal concentrations identified as being below detection limits are illustrated with a less than symbol ("<") and are plotted at the detection limit. The 90th centile of the distribution is designated with a solid horizontal blue line while a dashed line and a solid black line are used to identify the probably effect concentration and threshold effect concentration, respectively, if available.

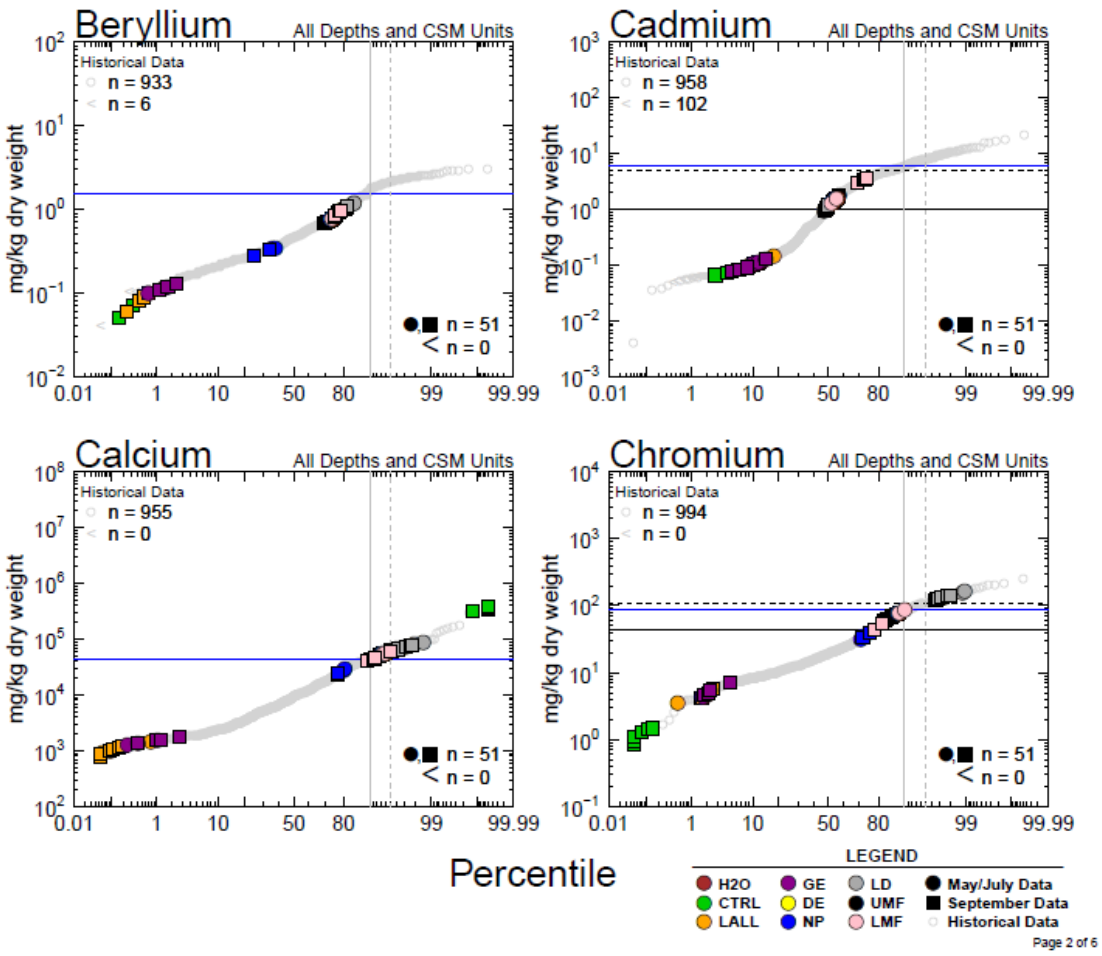


Figure D3.2. Bulk metal concentration in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Metal concentrations identified as being below detection limits are illustrated with a less than symbol ("<") and are plotted at the detection limit. The 90th centile of the distribution is designated with a solid horizontal blue line while a dashed line and a solid black line are used to identify the probably effect concentration and threshold effect concentration, respectively, if available.

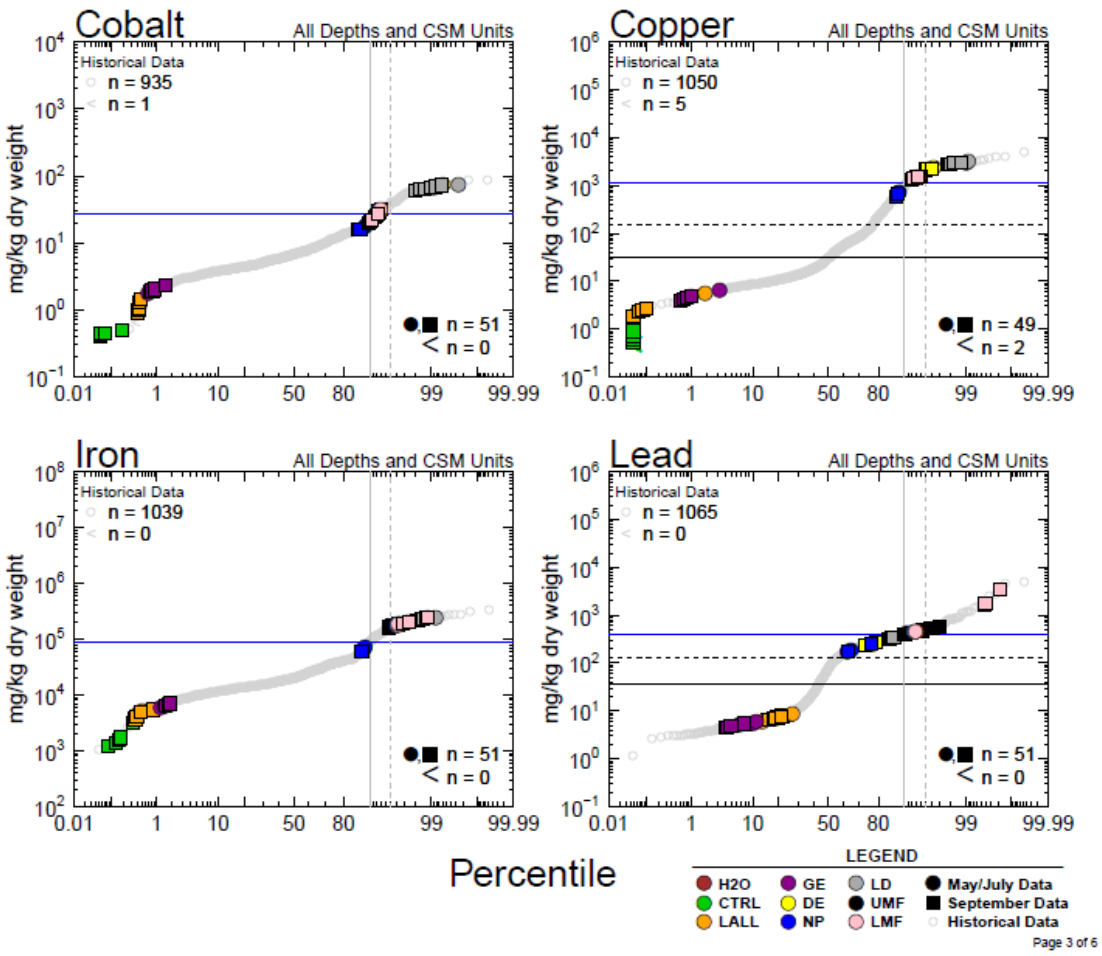


Figure D3.3. Bulk metal concentration in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Metal concentrations identified as being below detection limits are illustrated with a less than symbol ("<") and are plotted at the detection limit. The 90th centile of the distribution is designated with a solid horizontal blue line while a dashed line and a solid black line are used to identify the probably effect concentration and threshold effect concentration, respectively, if available.

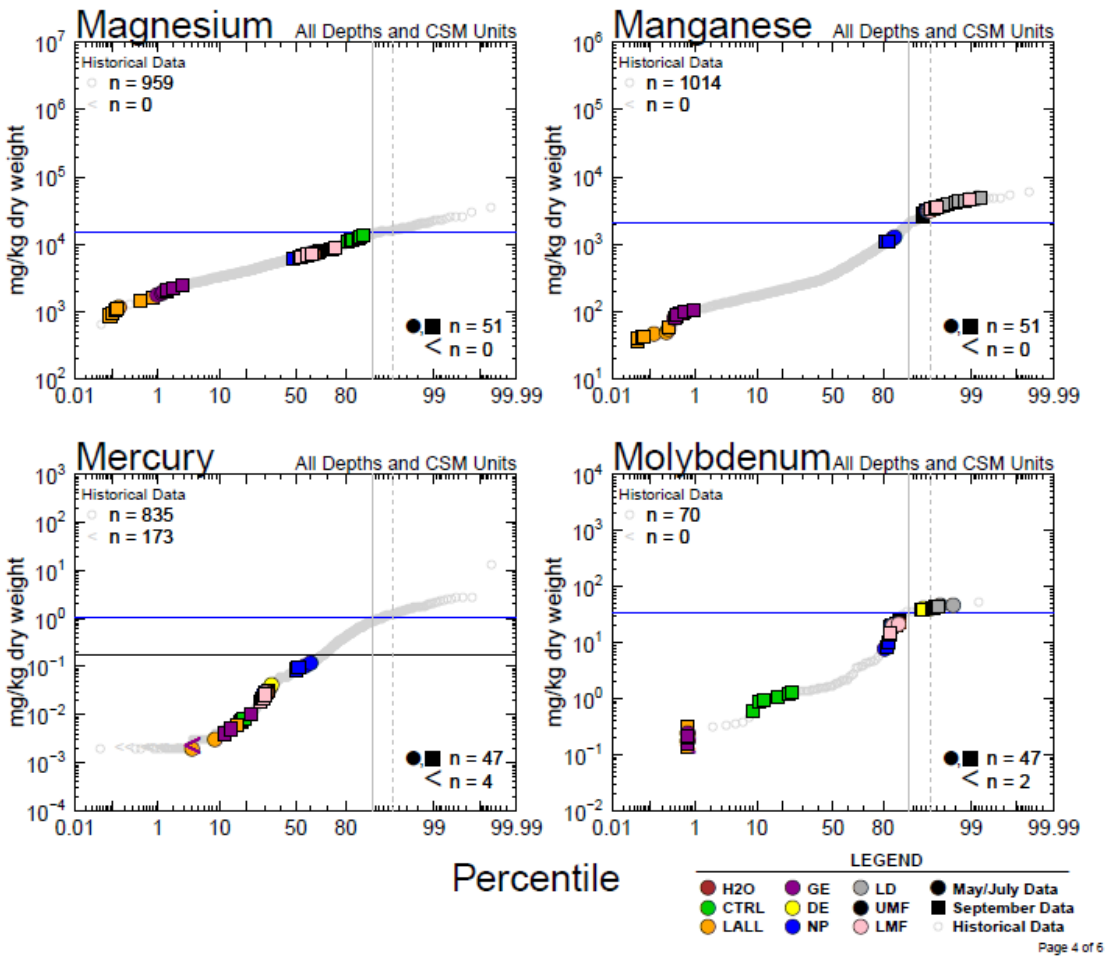


Figure D3.4. Bulk metal concentration in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Metal concentrations identified as being below detection limits are illustrated with a less than symbol ("<") and are plotted at the detection limit. The 90th centile of the distribution is designated with a solid horizontal blue line while a dashed line and a solid black line are used to identify the probably effect concentration and threshold effect concentration, respectively, if available.

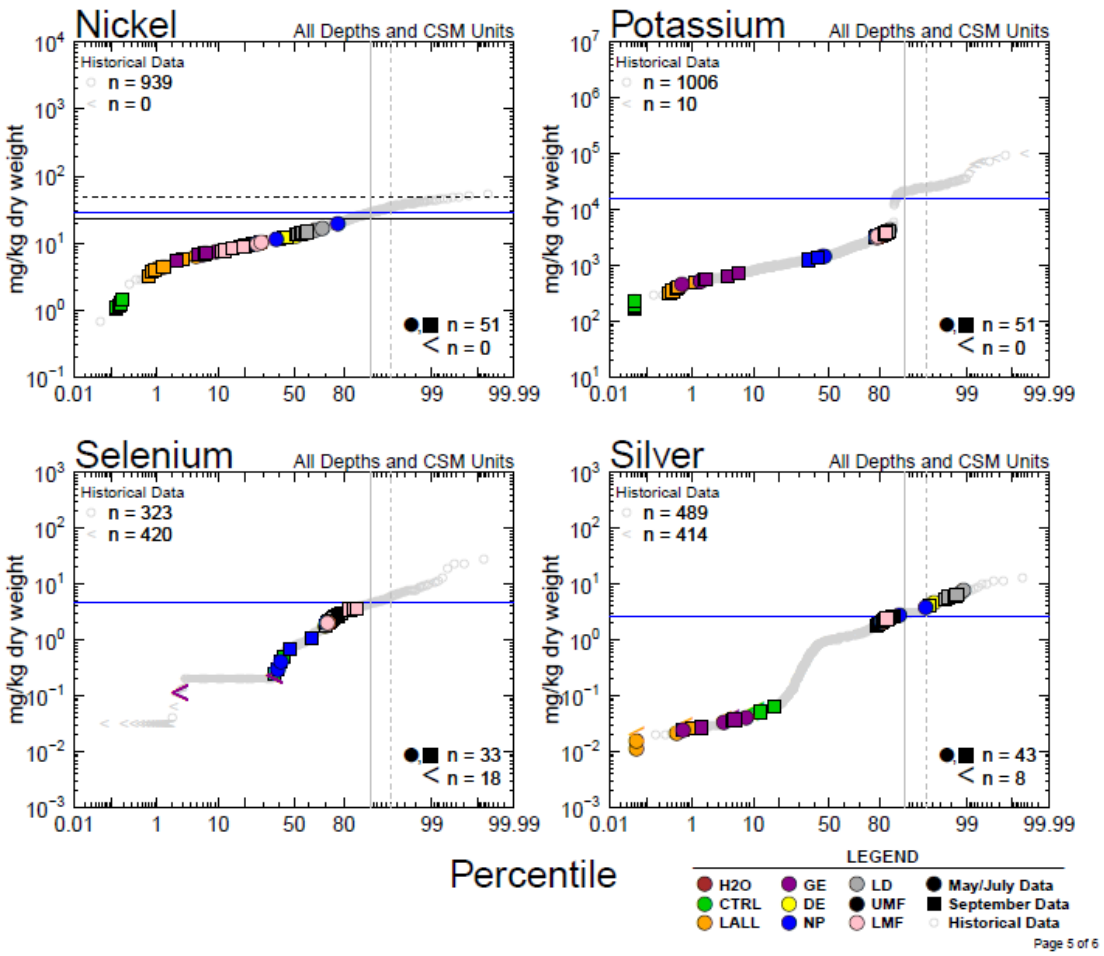


Figure D3.5. Bulk metal concentration in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Metal concentrations identified as being below detection limits are illustrated with a less than symbol ("<") and are plotted at the detection limit. The 90th centile of the distribution is designated with a solid horizontal blue line while a dashed line and a solid black line are used to identify the probably effect concentration and threshold effect concentration, respectively, if available.

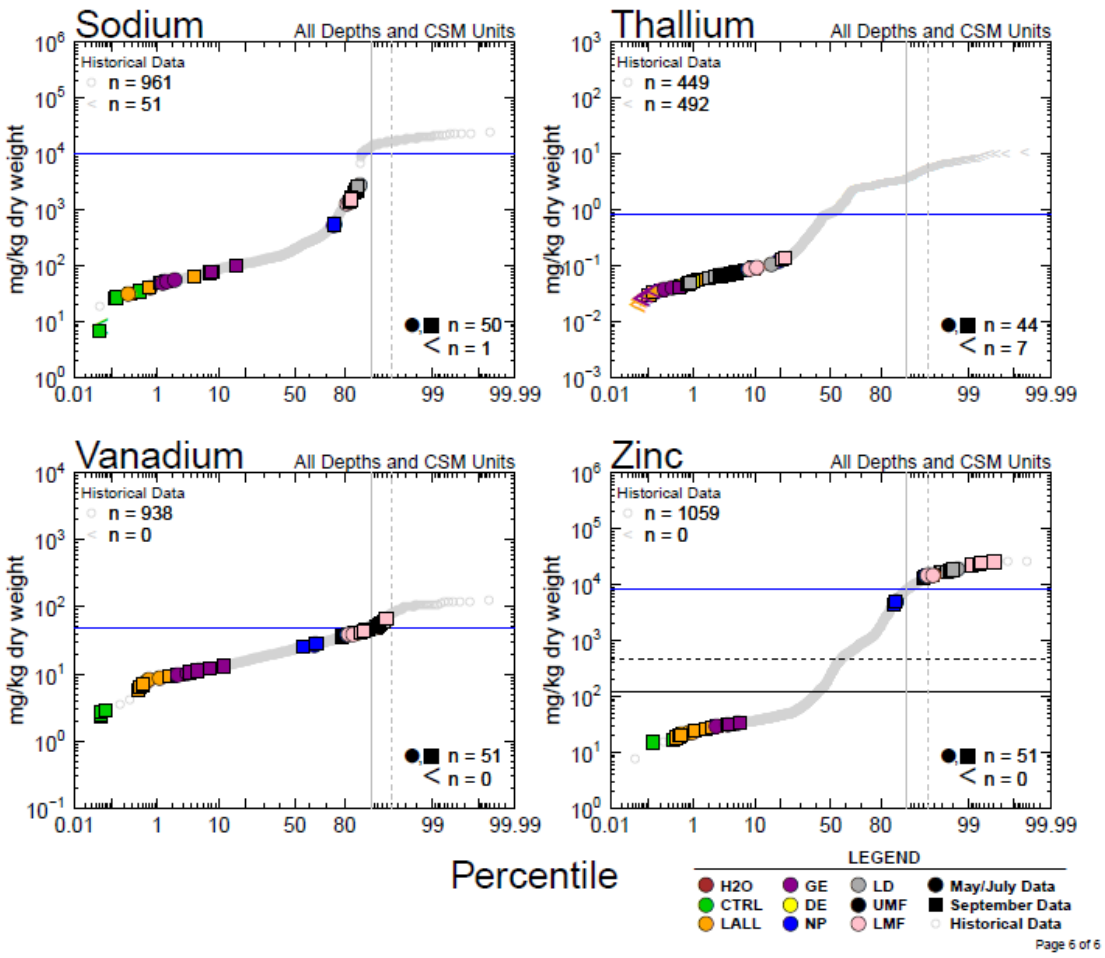


Figure D3.6. Bulk metal concentration in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Metal concentrations identified as being below detection limits are illustrated with a less than symbol ("<") and are plotted at the detection limit. The 90th centile of the distribution is designated with a solid horizontal blue line while a dashed line and a solid black line are used to identify the probably effect concentration and threshold effect concentration, respectively, if available.

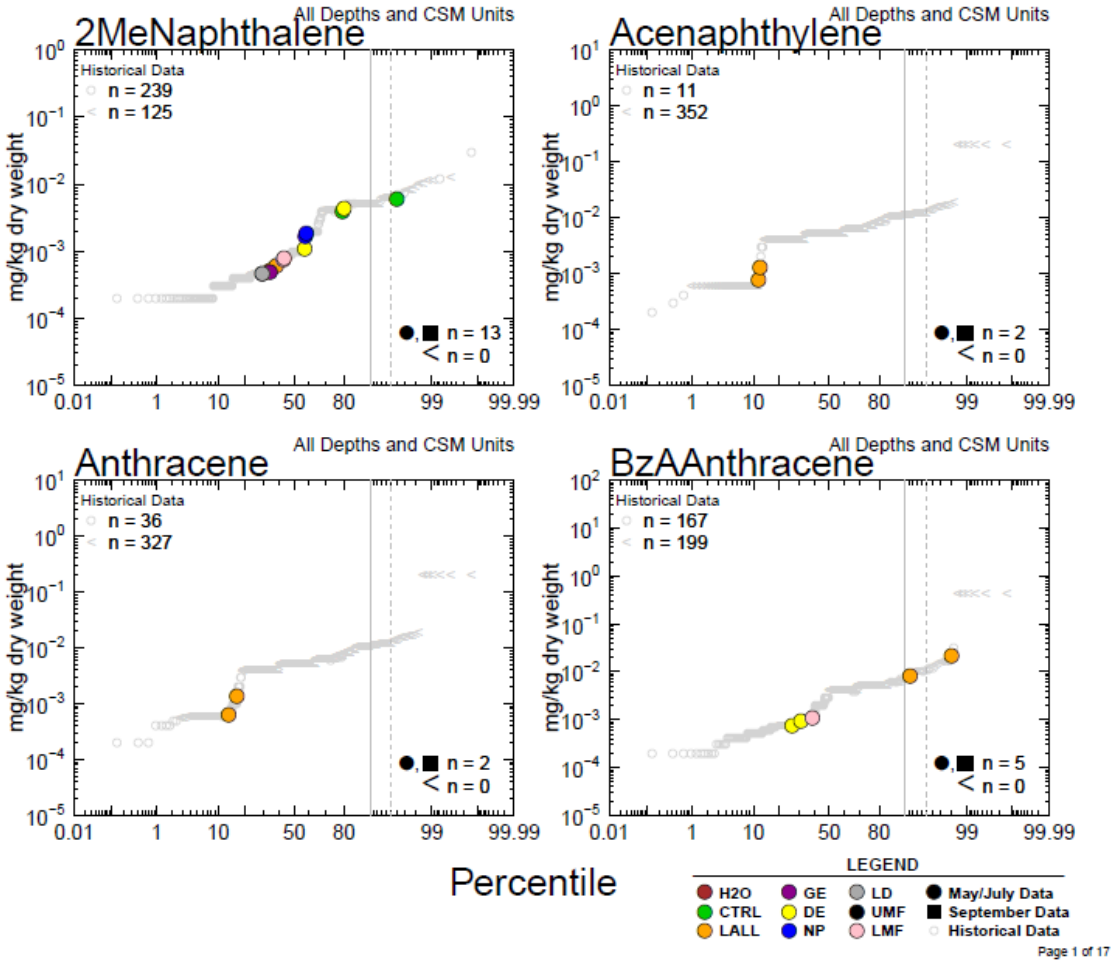


Figure D3.7. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

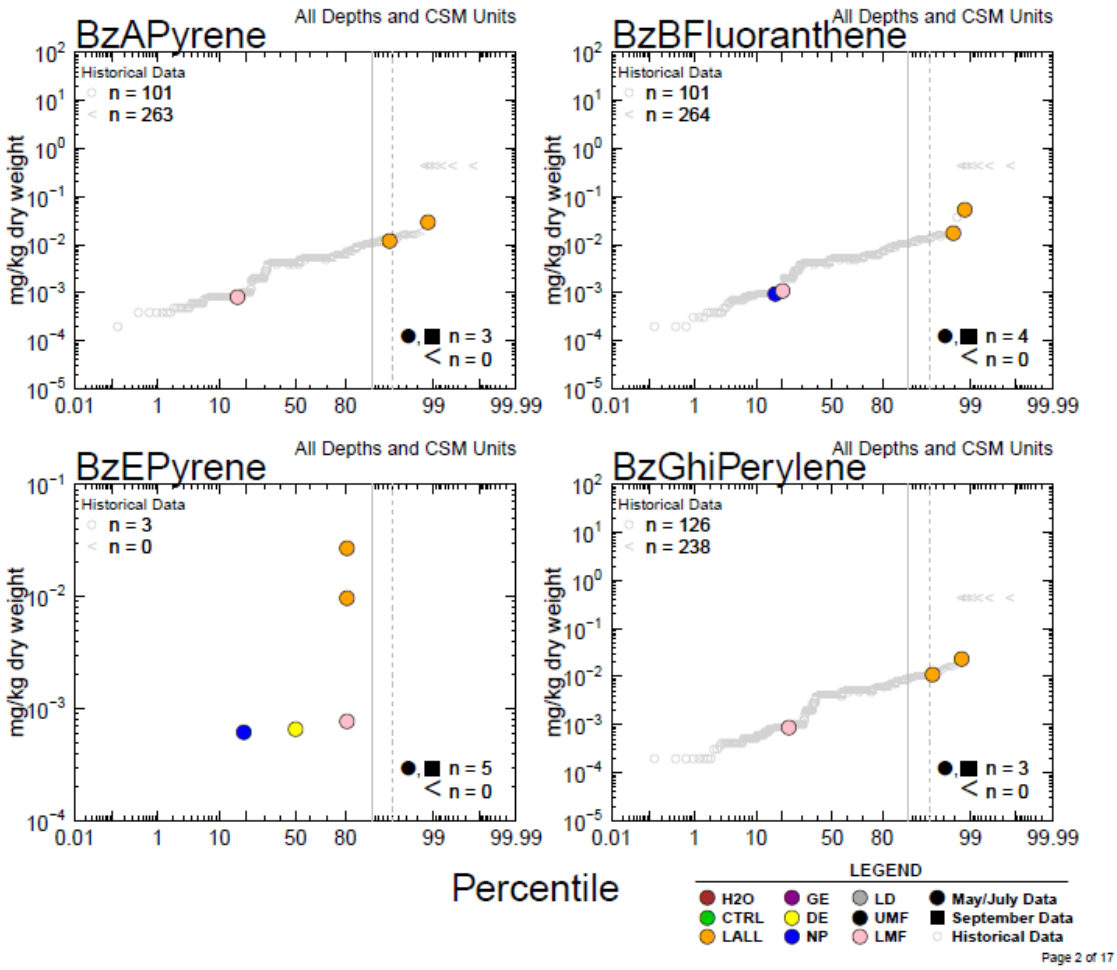


Figure D3.8. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

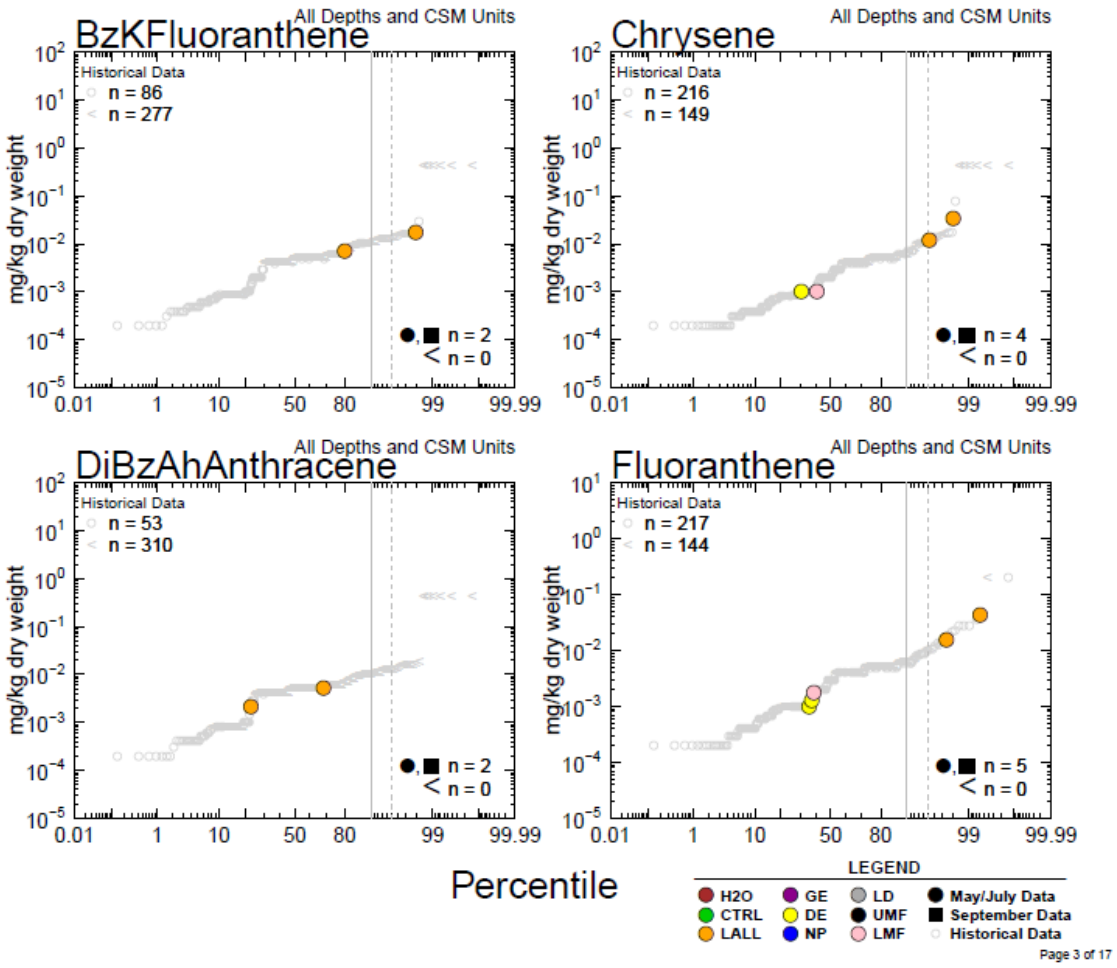


Figure D3.9. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

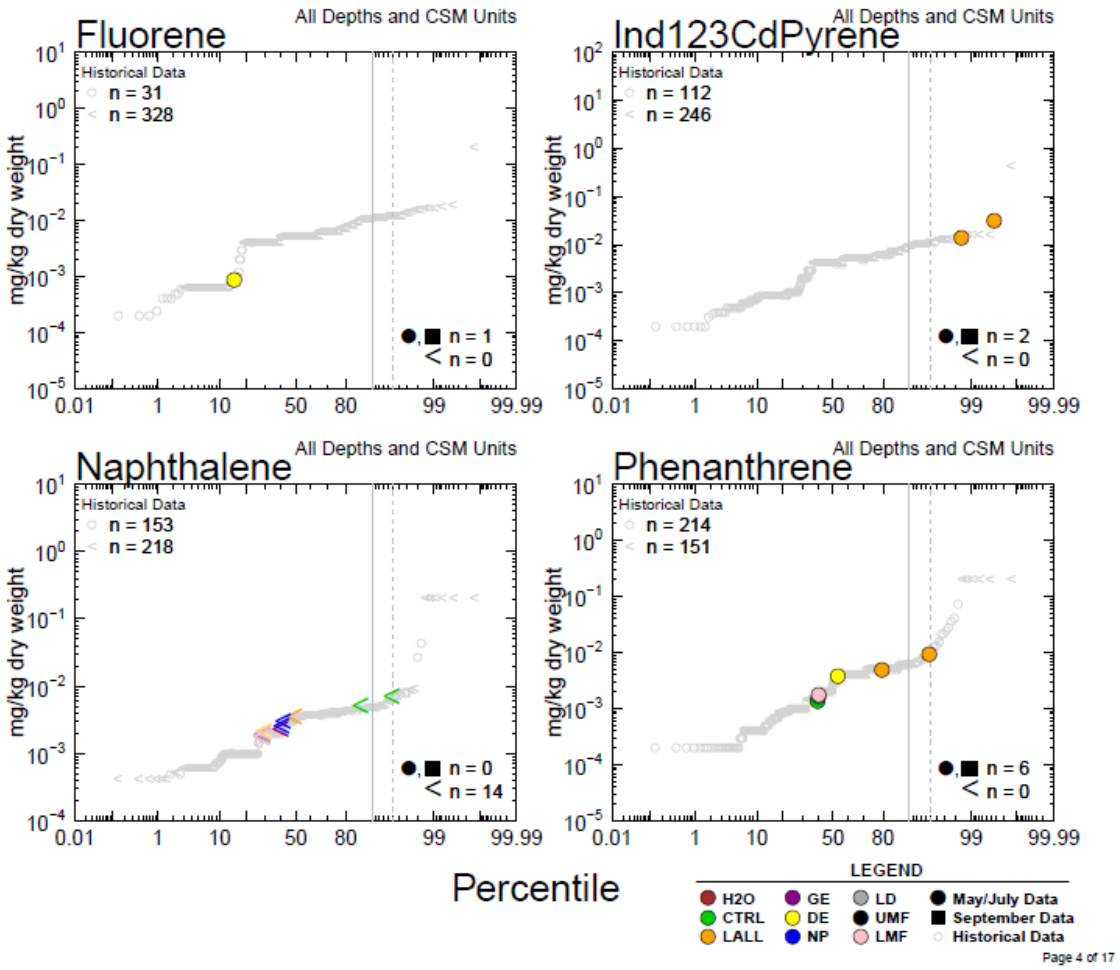


Figure D3.10. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

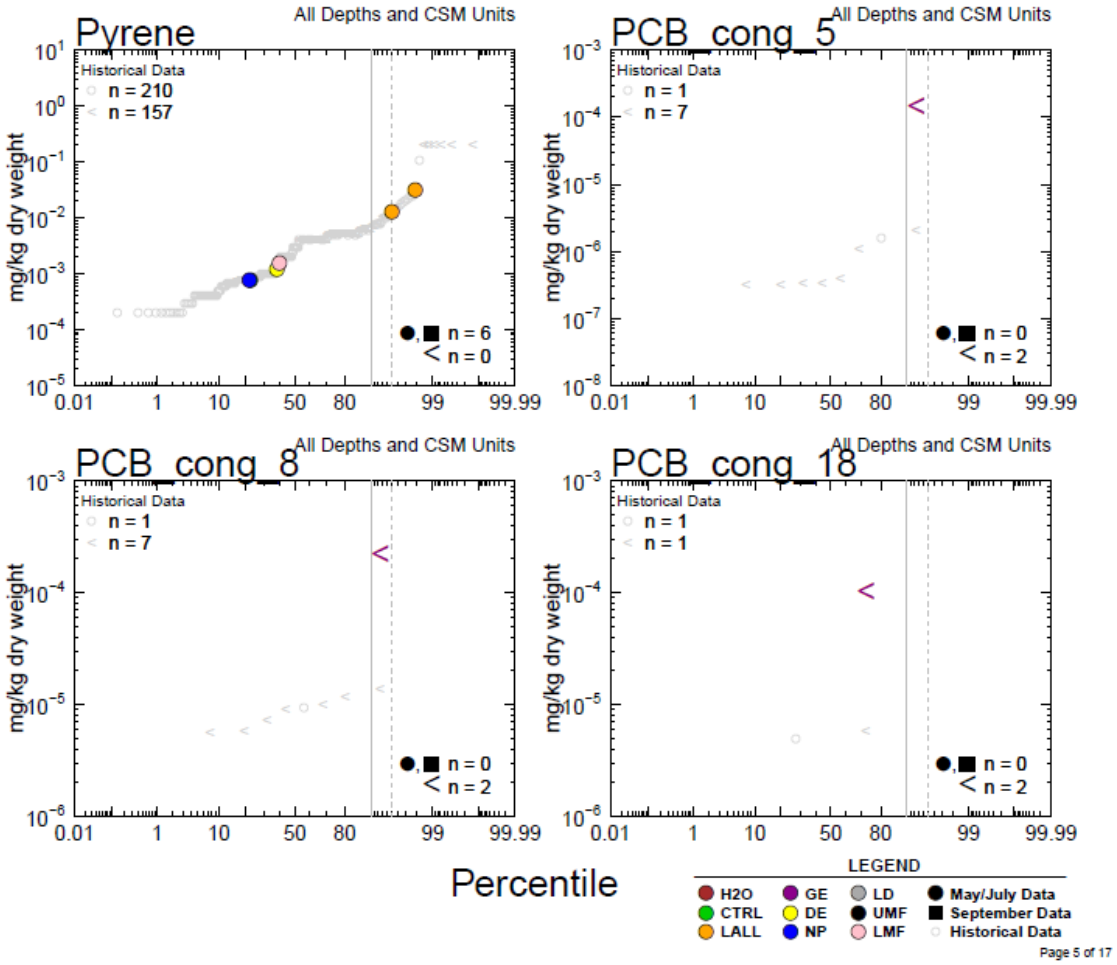


Figure D3.11. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

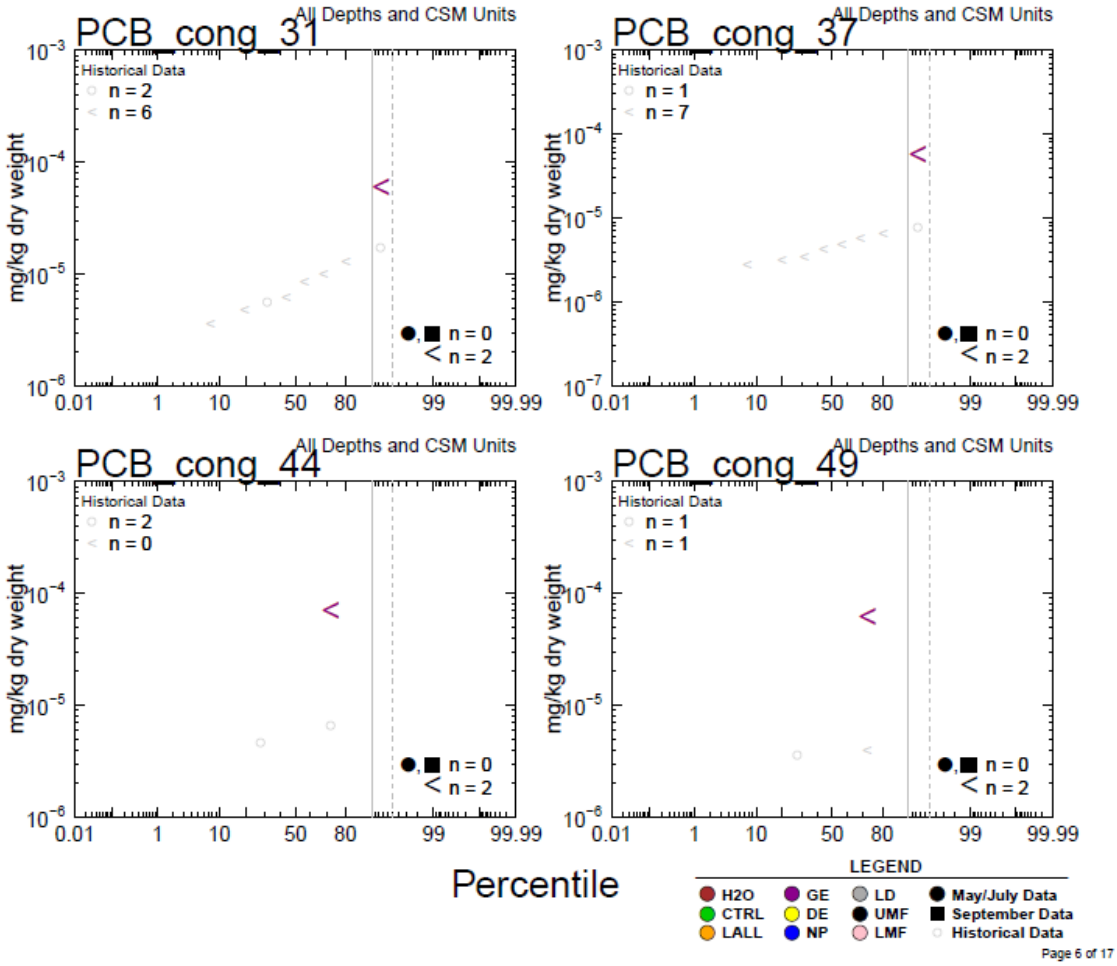


Figure D3.12. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

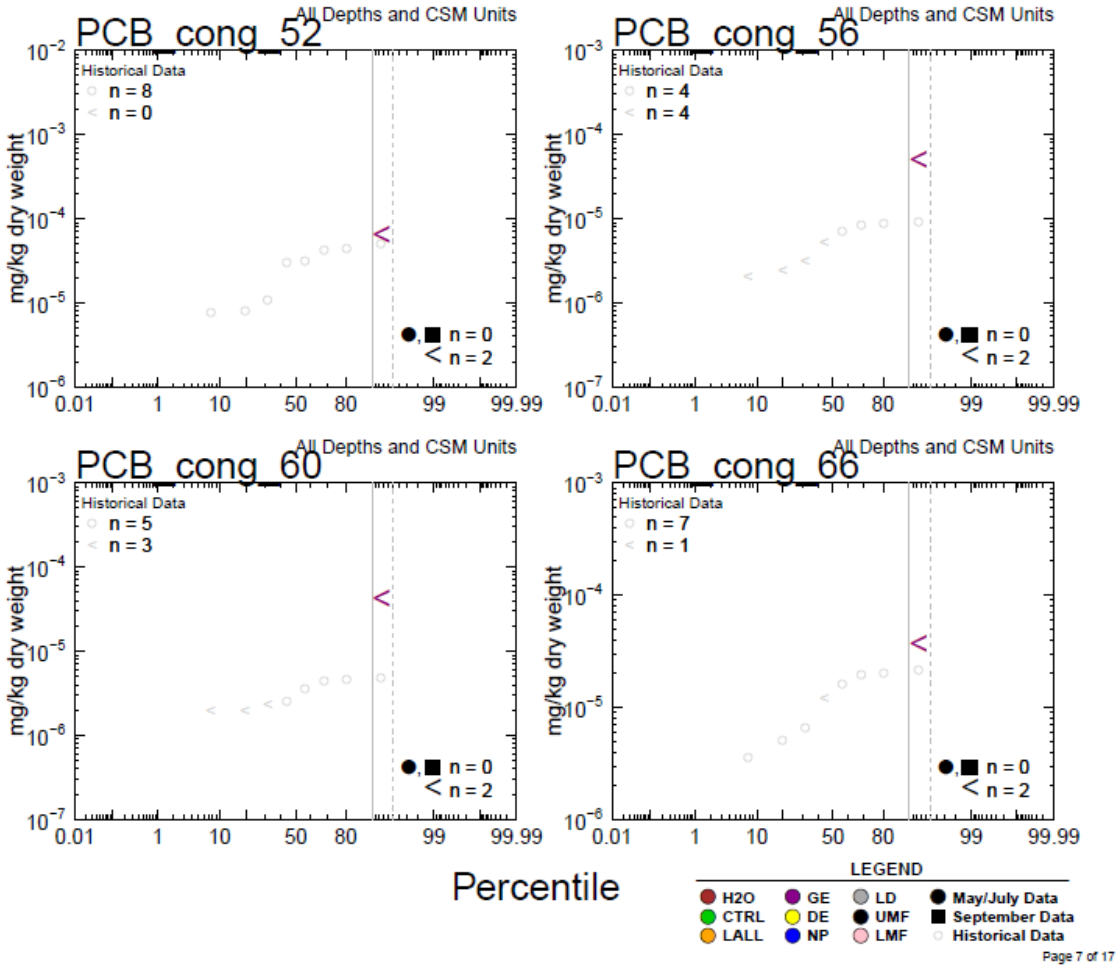


Figure D3.13. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

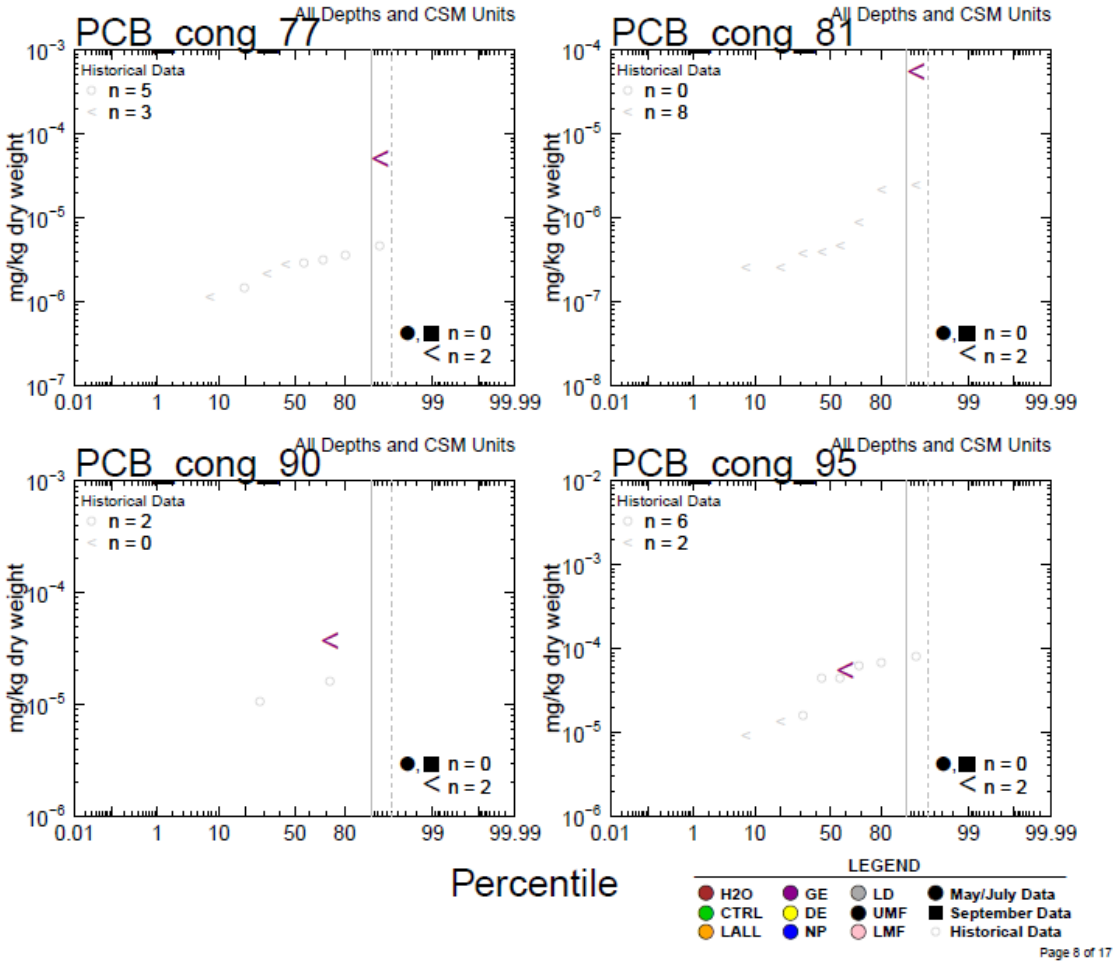


Figure D3.14. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

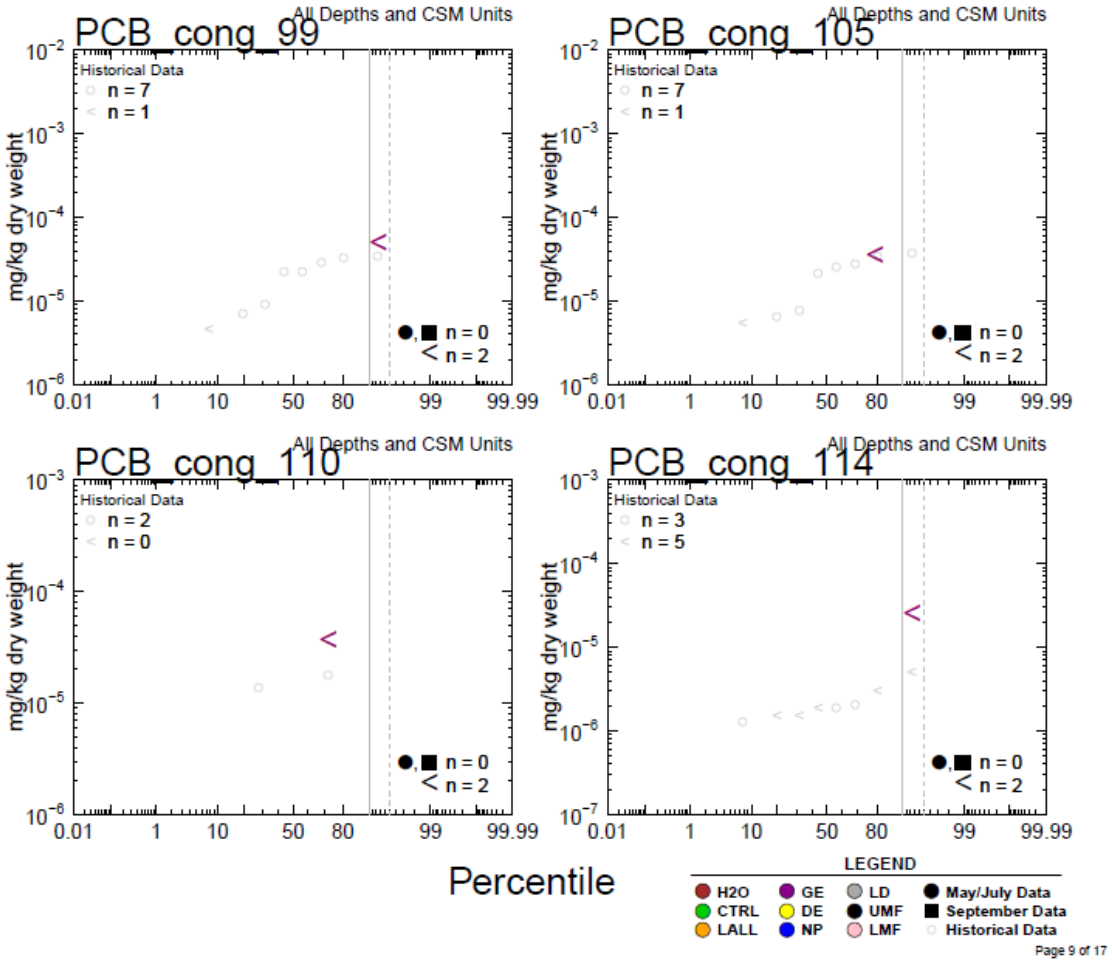


Figure D3.15. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

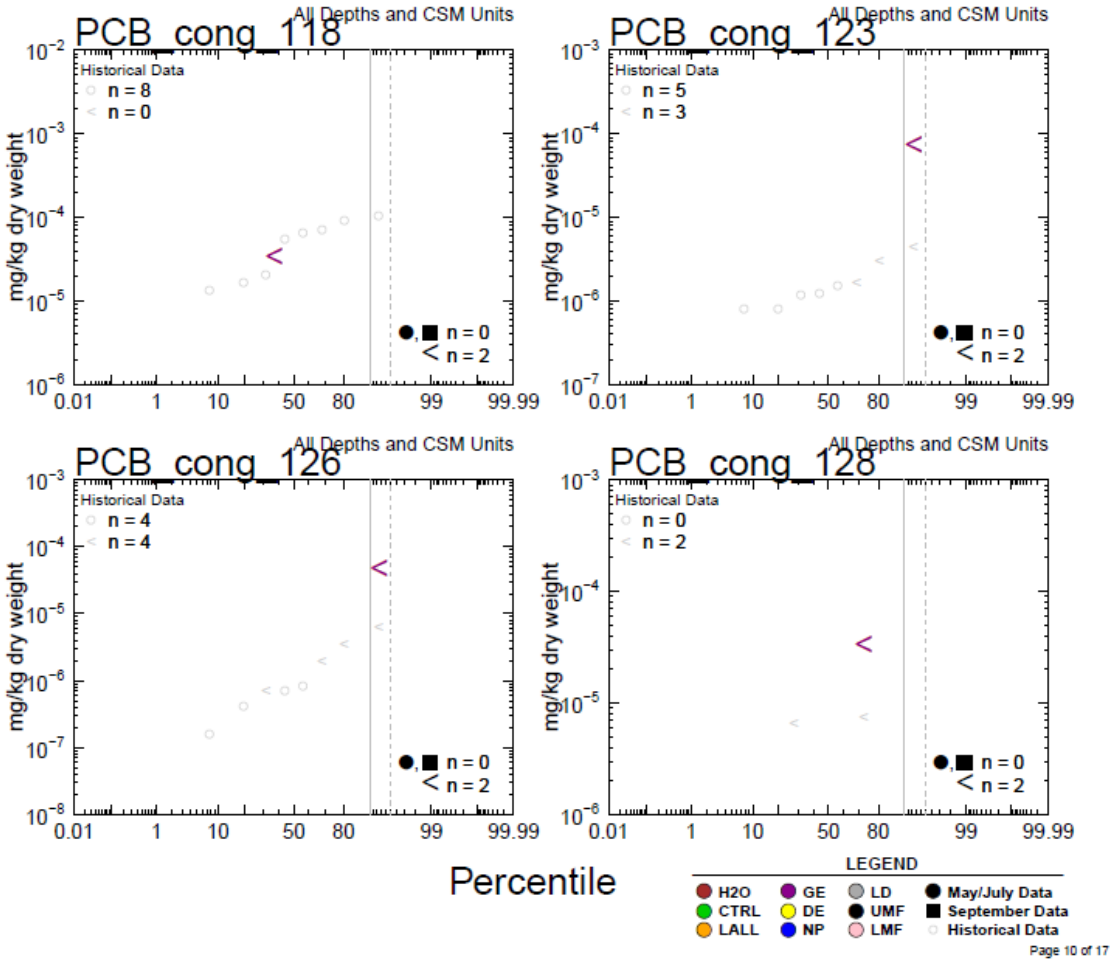


Figure D3.16. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

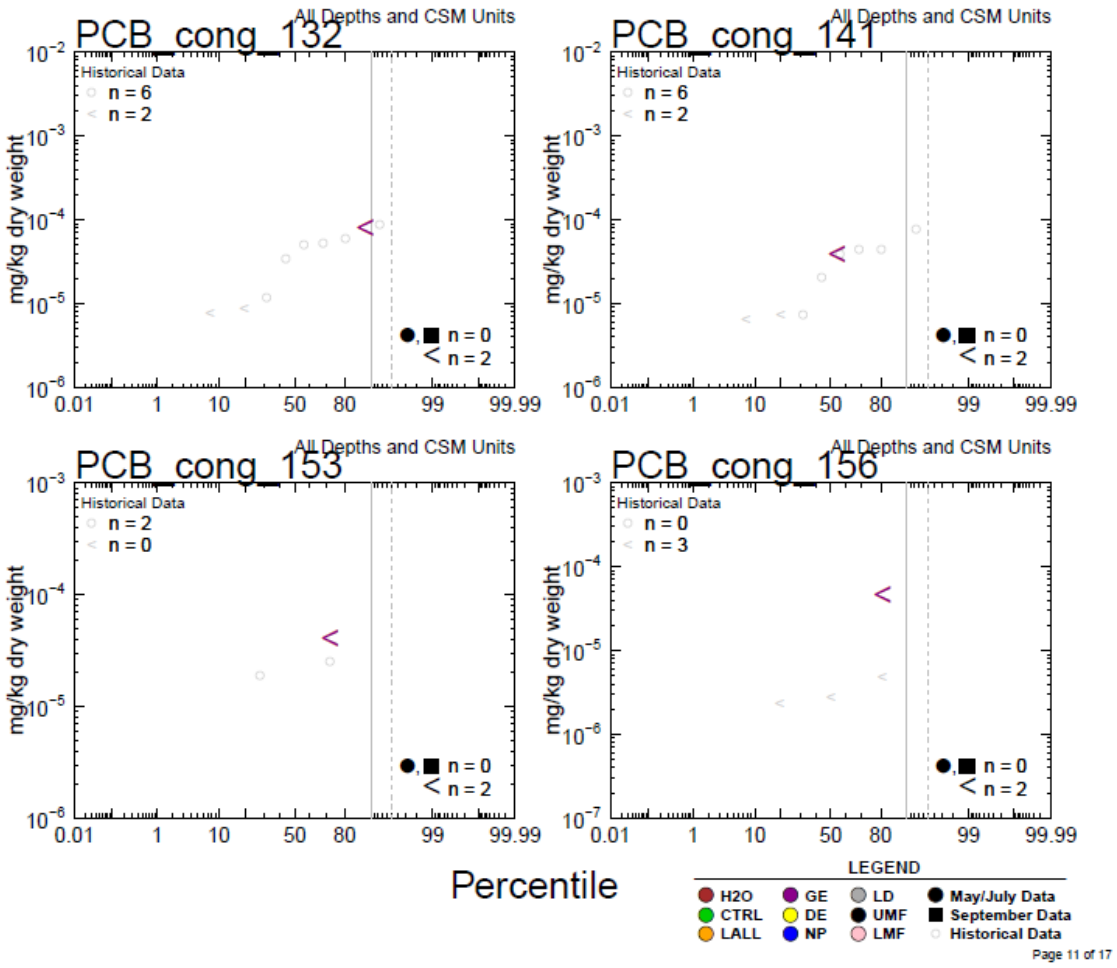


Figure D3.17. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

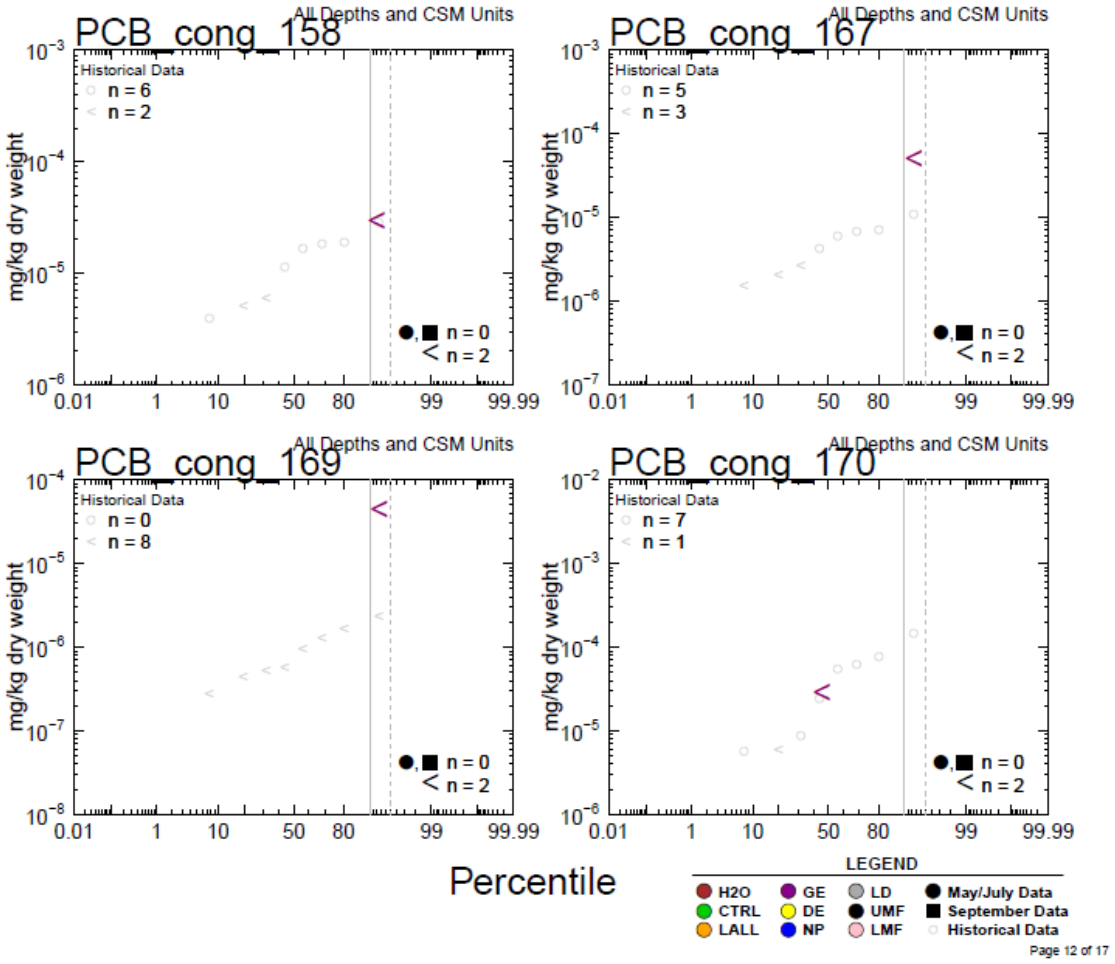


Figure D3.18. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

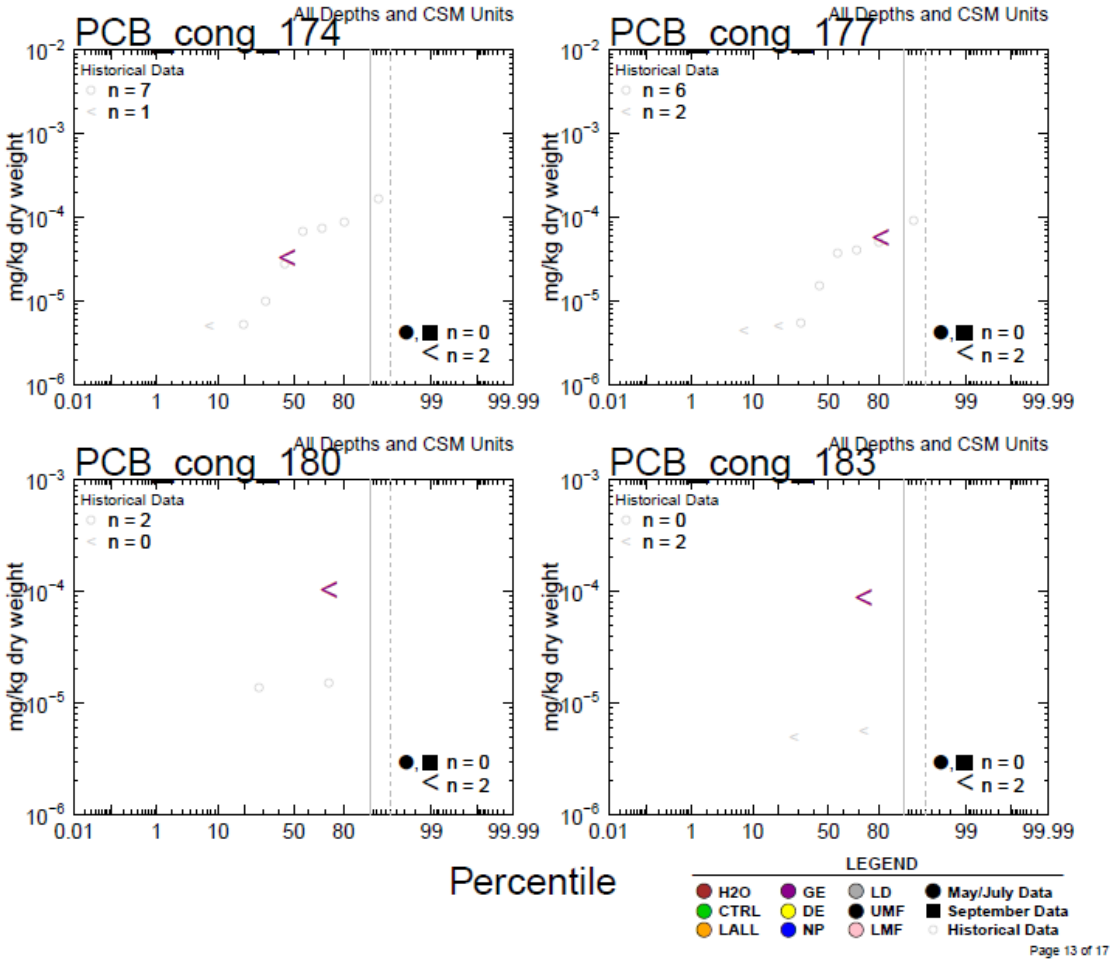


Figure D3.19. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

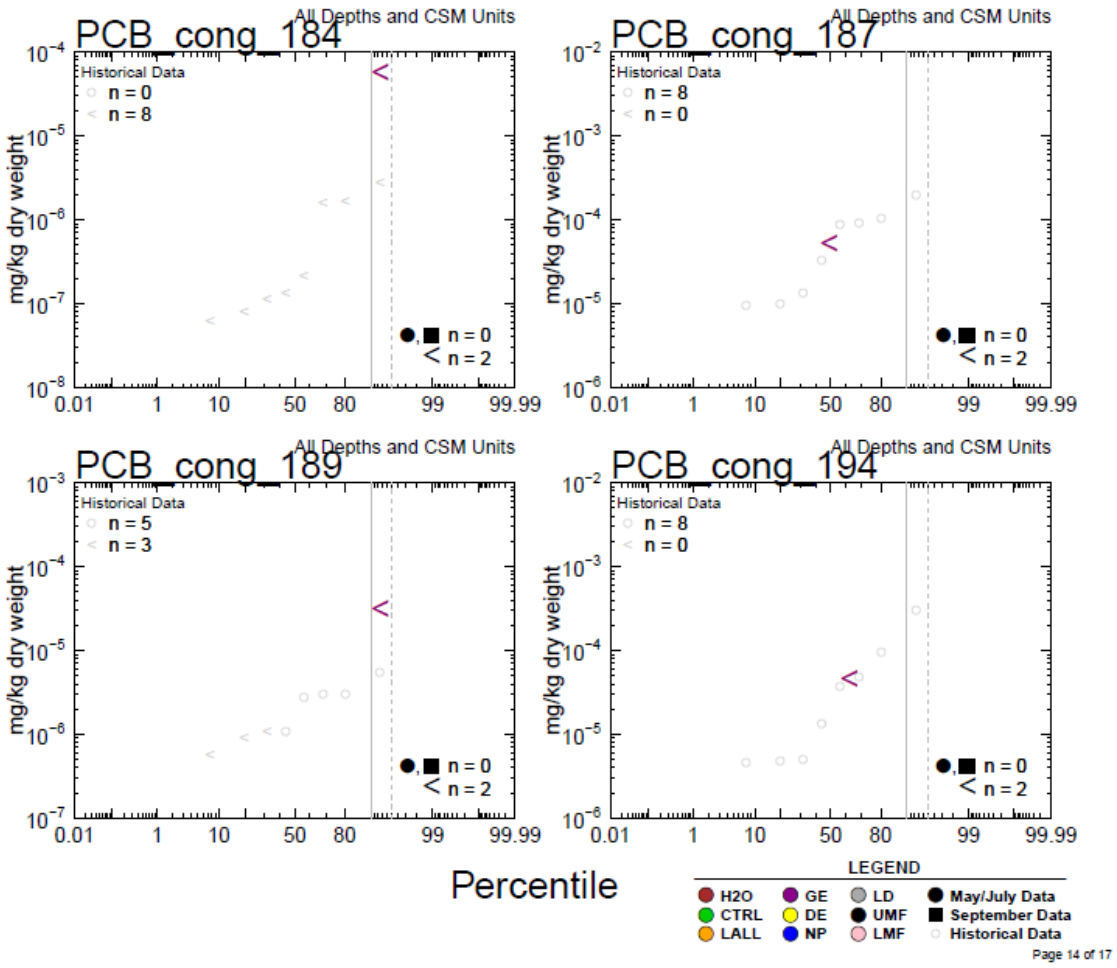


Figure D3.20. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

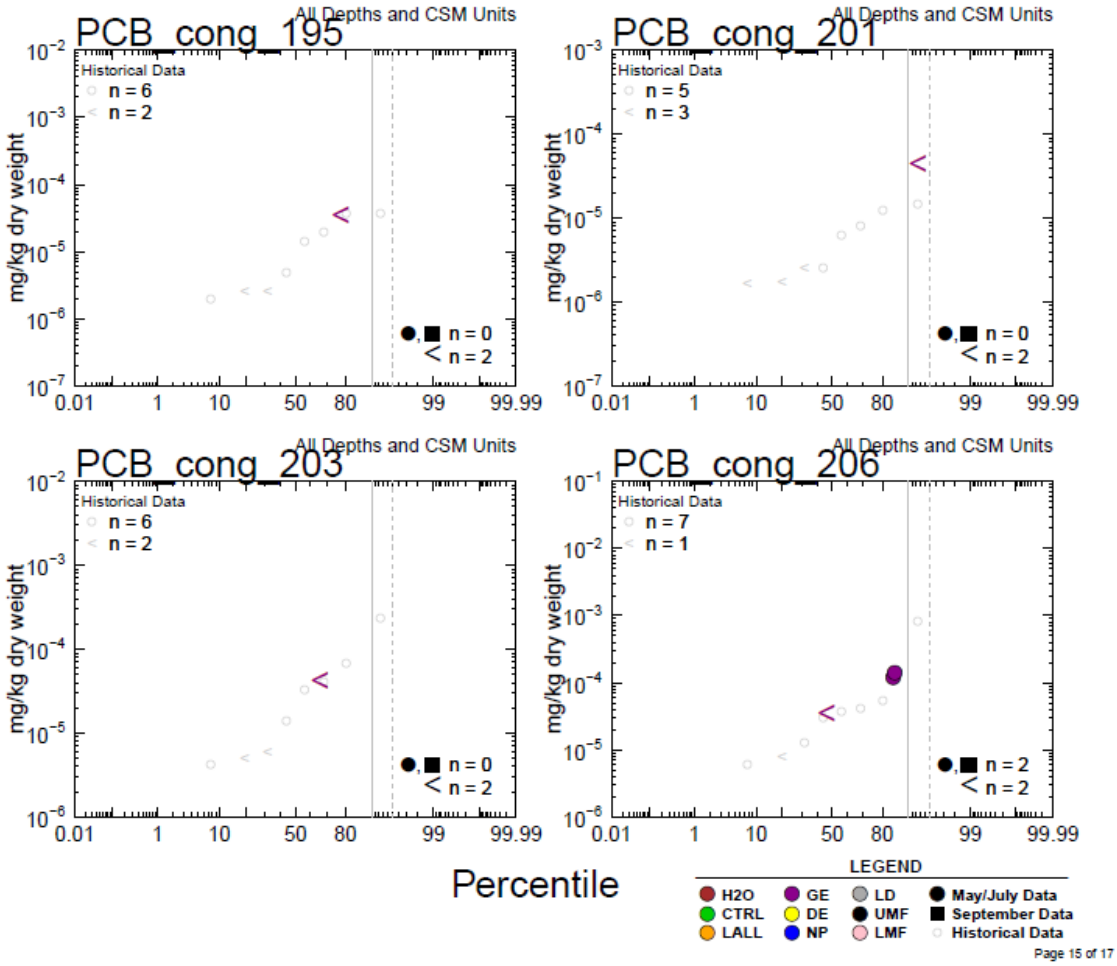


Figure D3.21. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

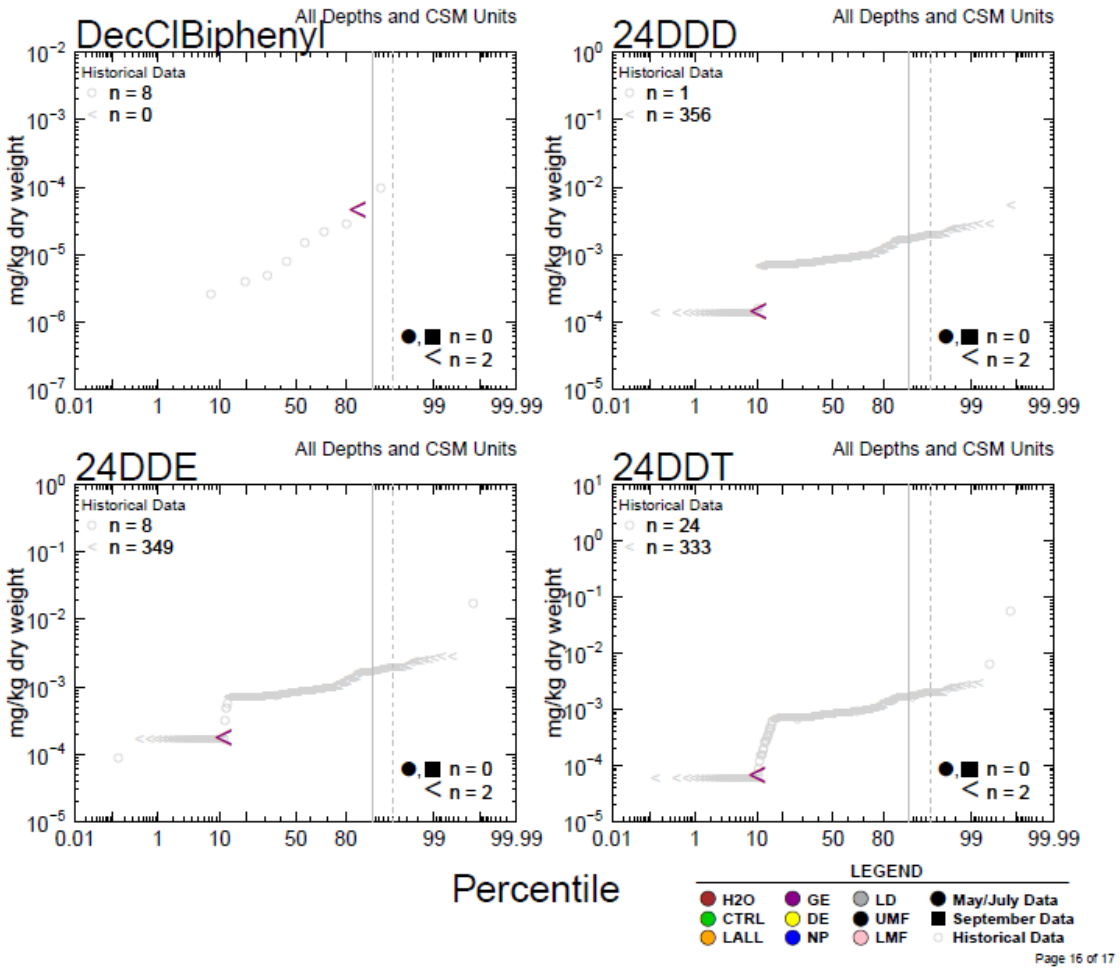


Figure D3.22. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

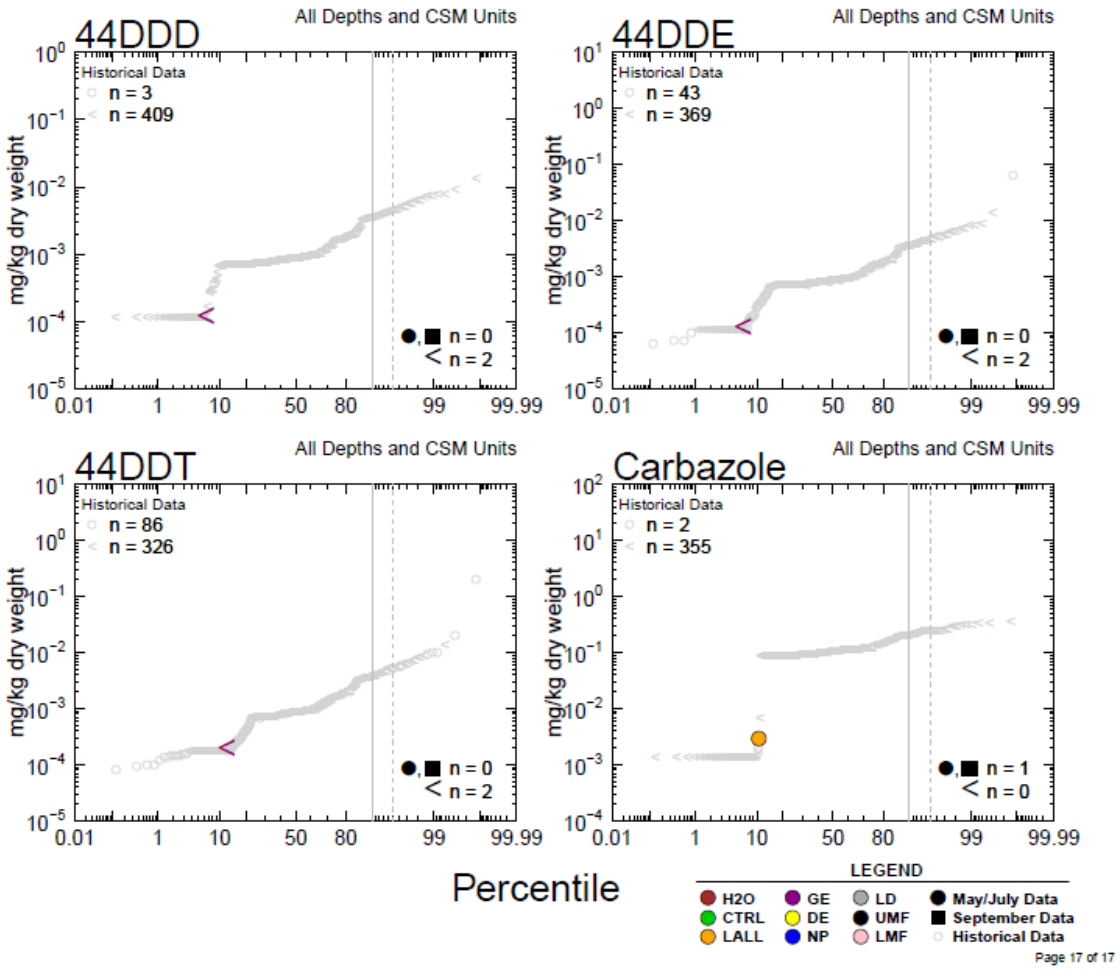


Figure D3.23. Bulk concentration of organic contaminant in sediment samples evaluated within the white sturgeon sediment toxicity tests.

Concentrations identified as being below detection limits are illustrated with a less than symbol (”<”) and are plotted at the detection limit.

APPENDIX D4

Box and whisker plots for concentrations of metal as a function of treatment and sample type for white sturgeon toxicity testing

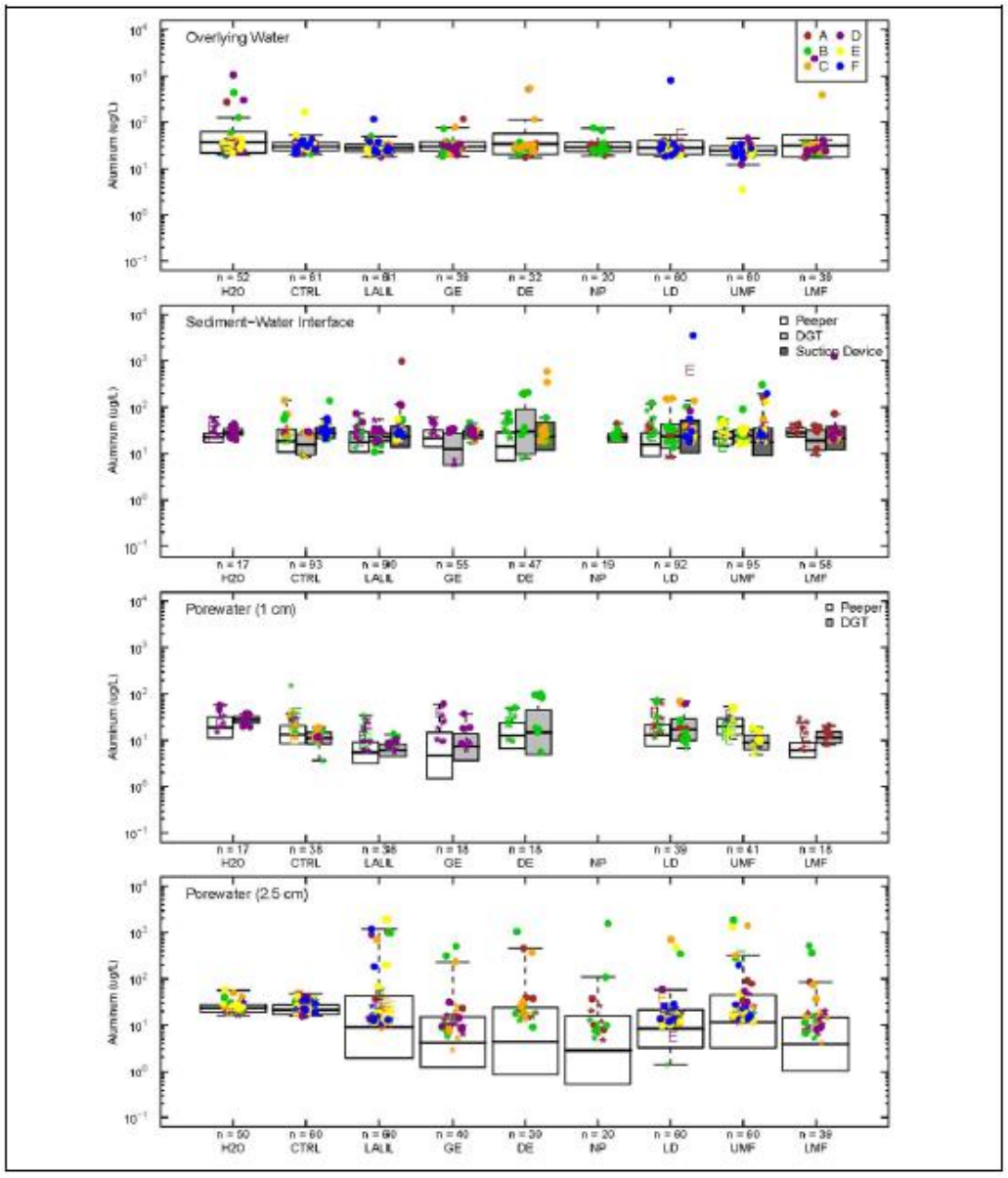


Figure D4.1. Concentrations of dissolved aluminum as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

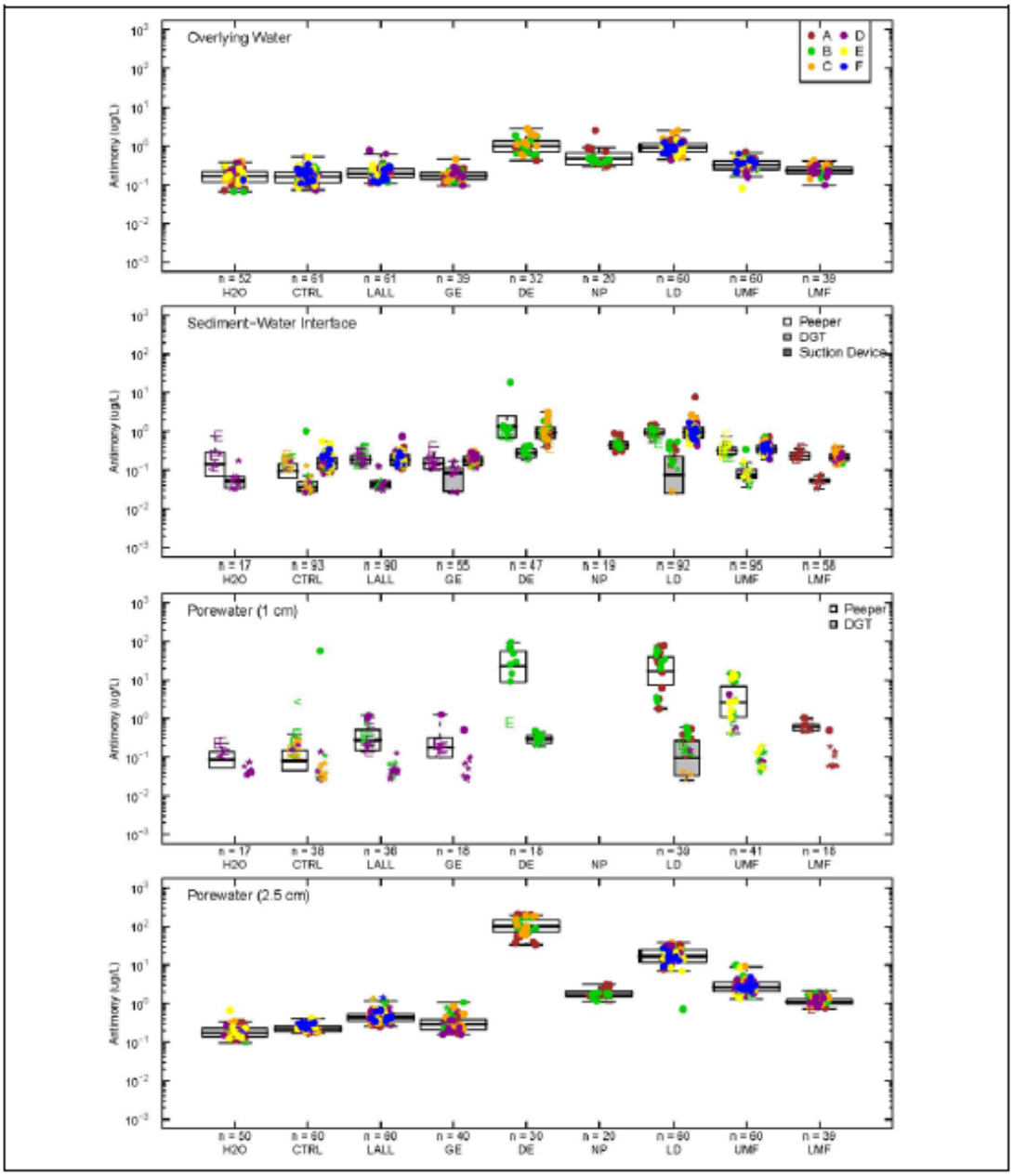


Figure D4.2. Concentrations of dissolved antimony as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

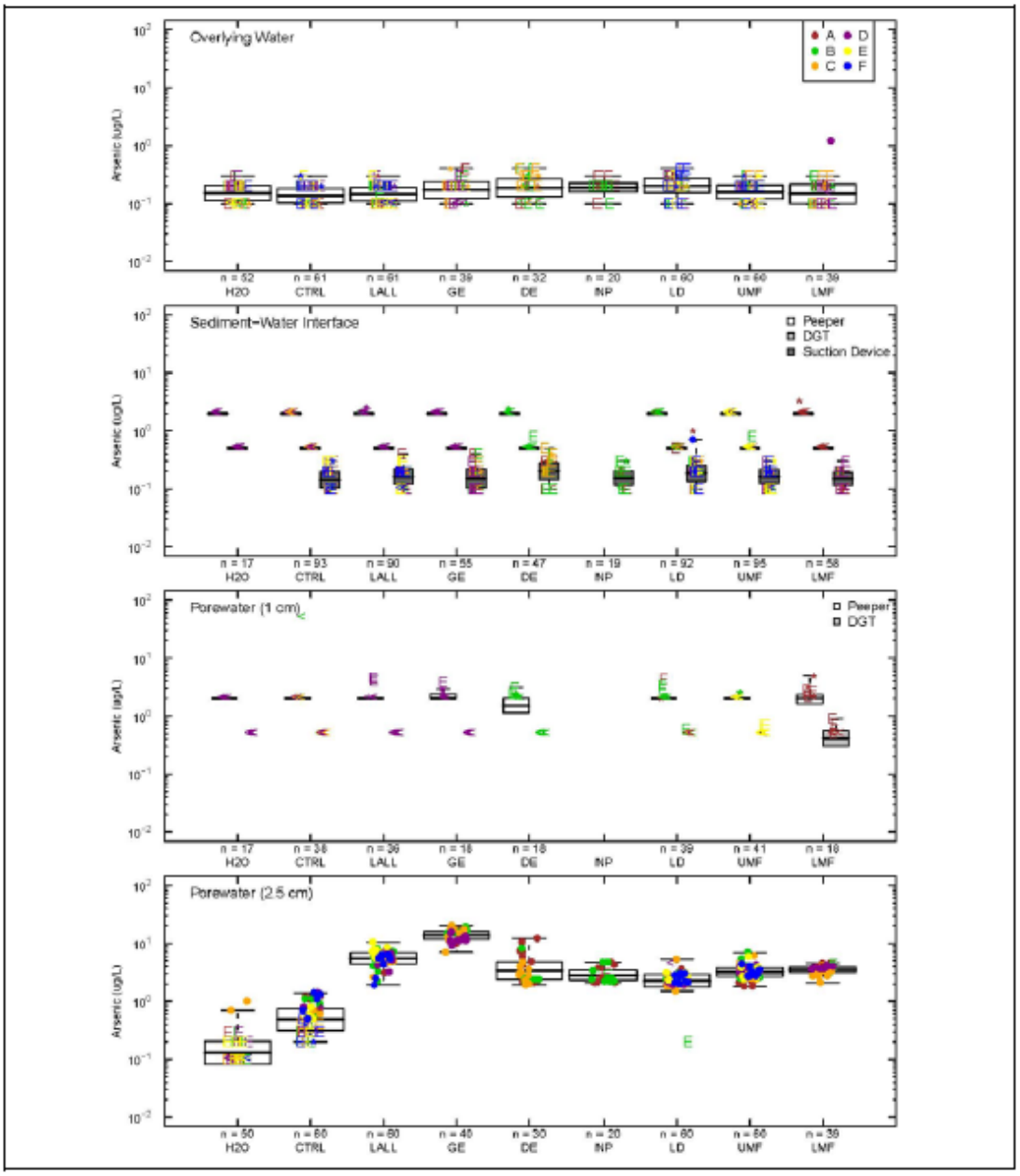


Figure D4.3. Concentrations of dissolved arsenic as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

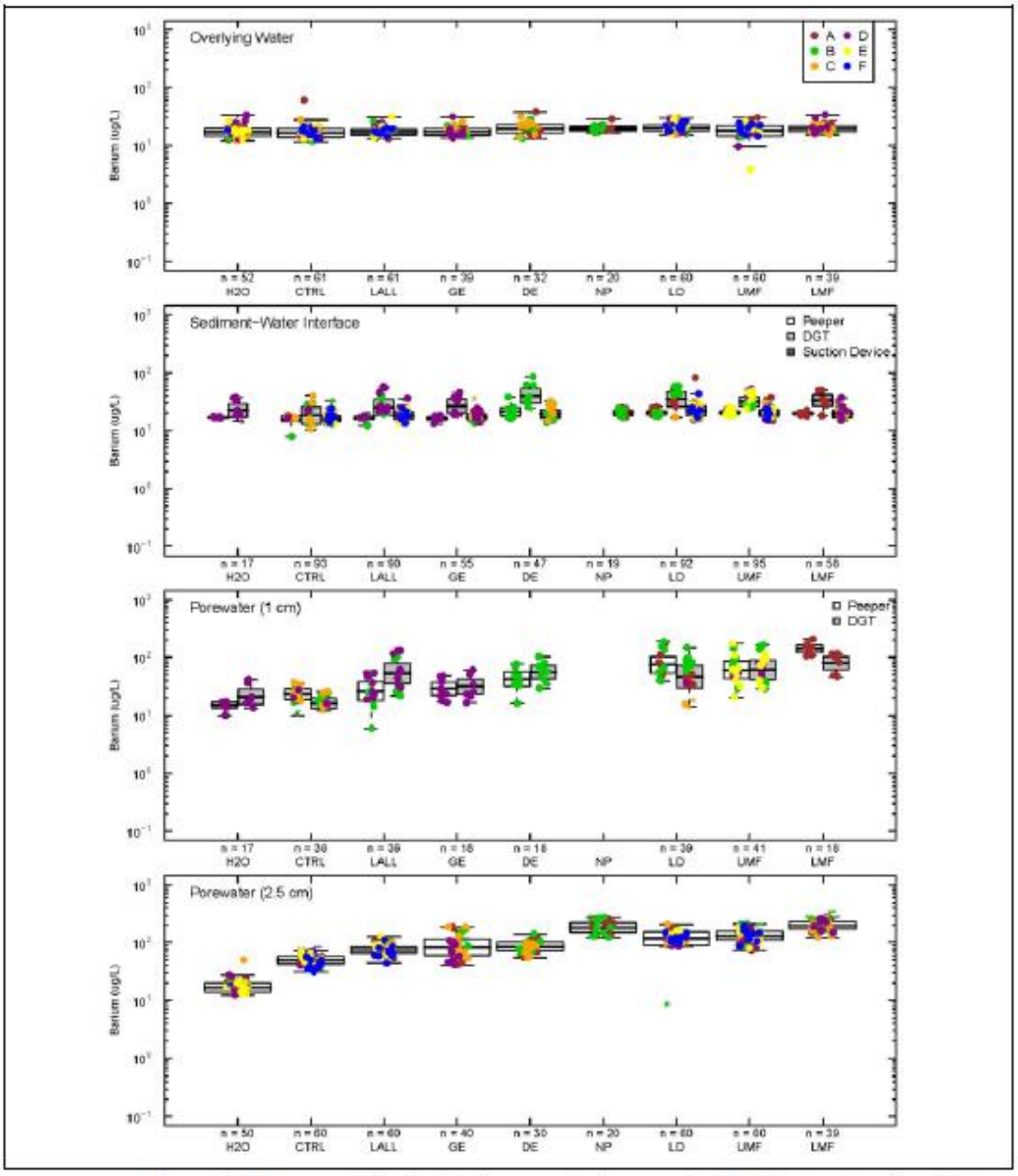


Figure D4.4. Concentrations of dissolved barium as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

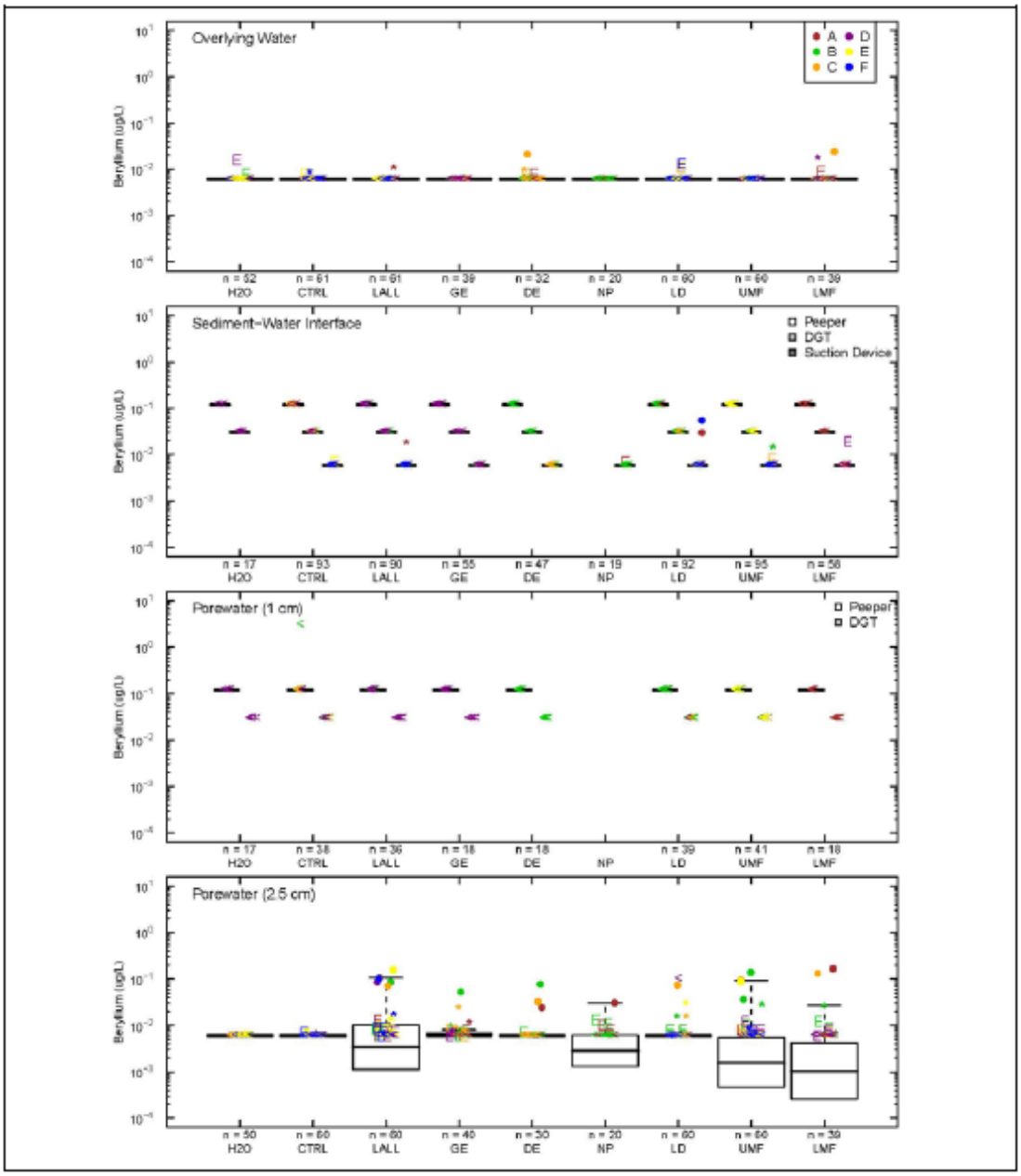


Figure D4.5. Concentrations of dissolved beryllium as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

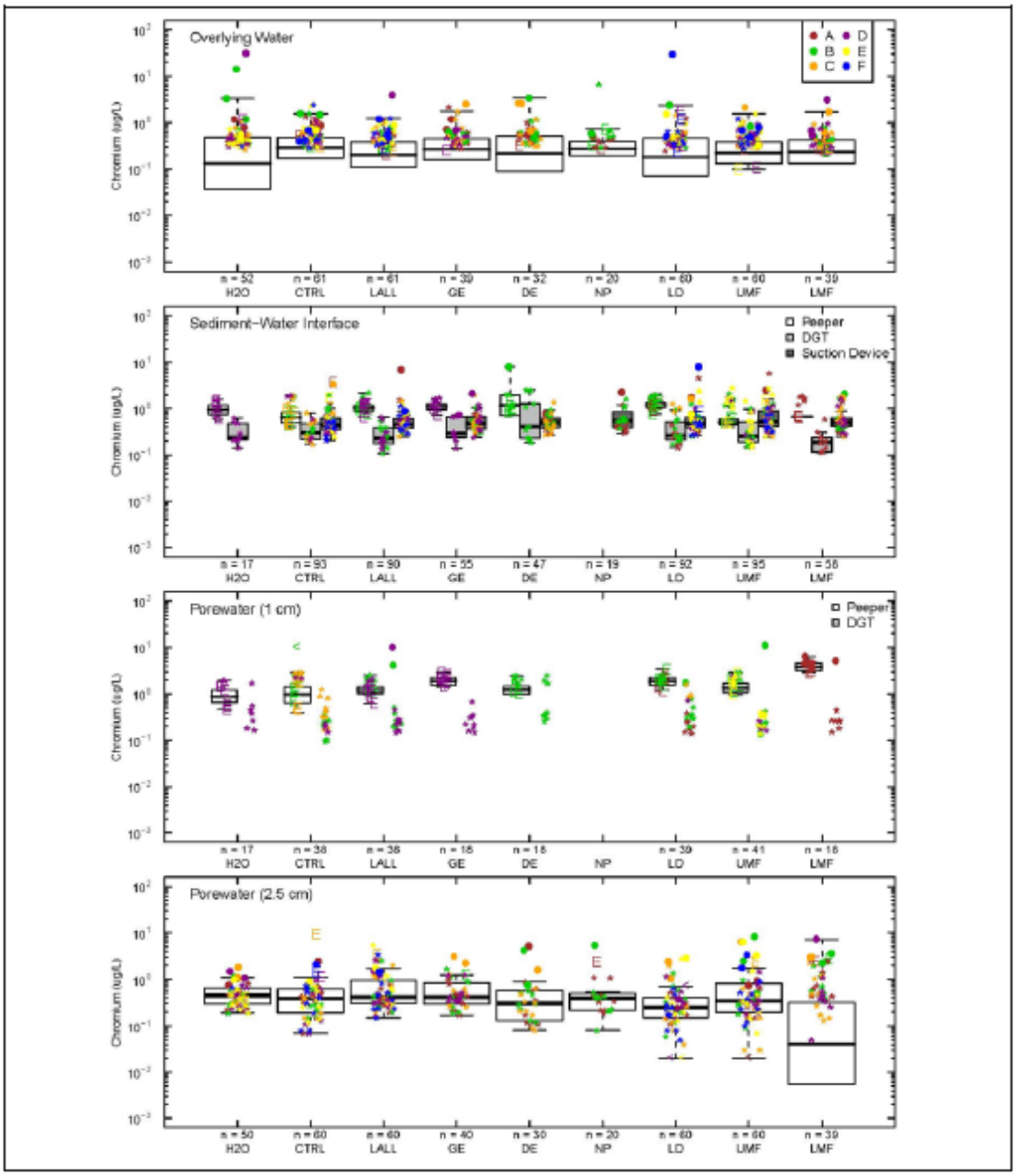


Figure D4.6. Concentrations of dissolved chromium as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

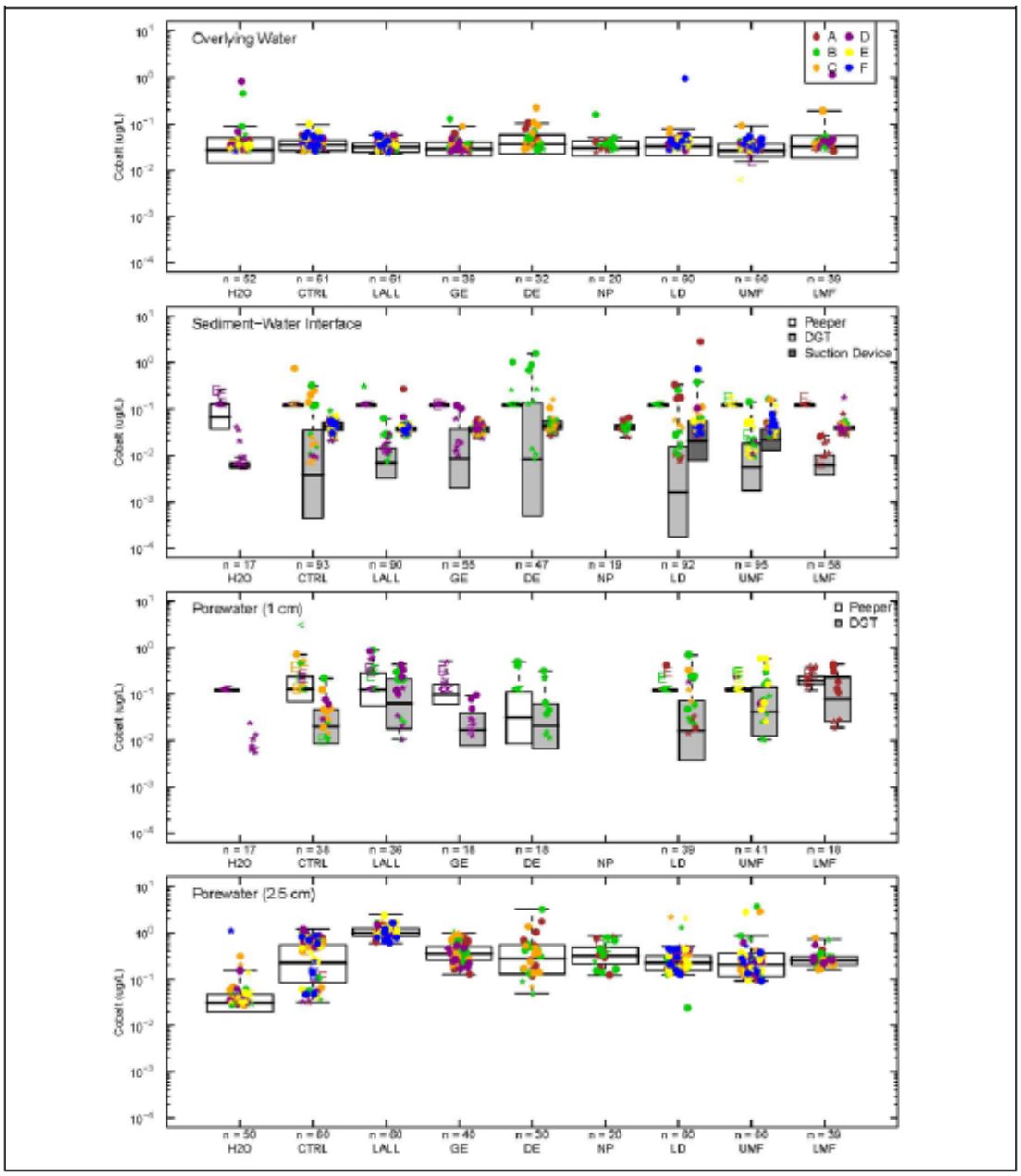


Figure D4.7. Concentrations of dissolved cobalt as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

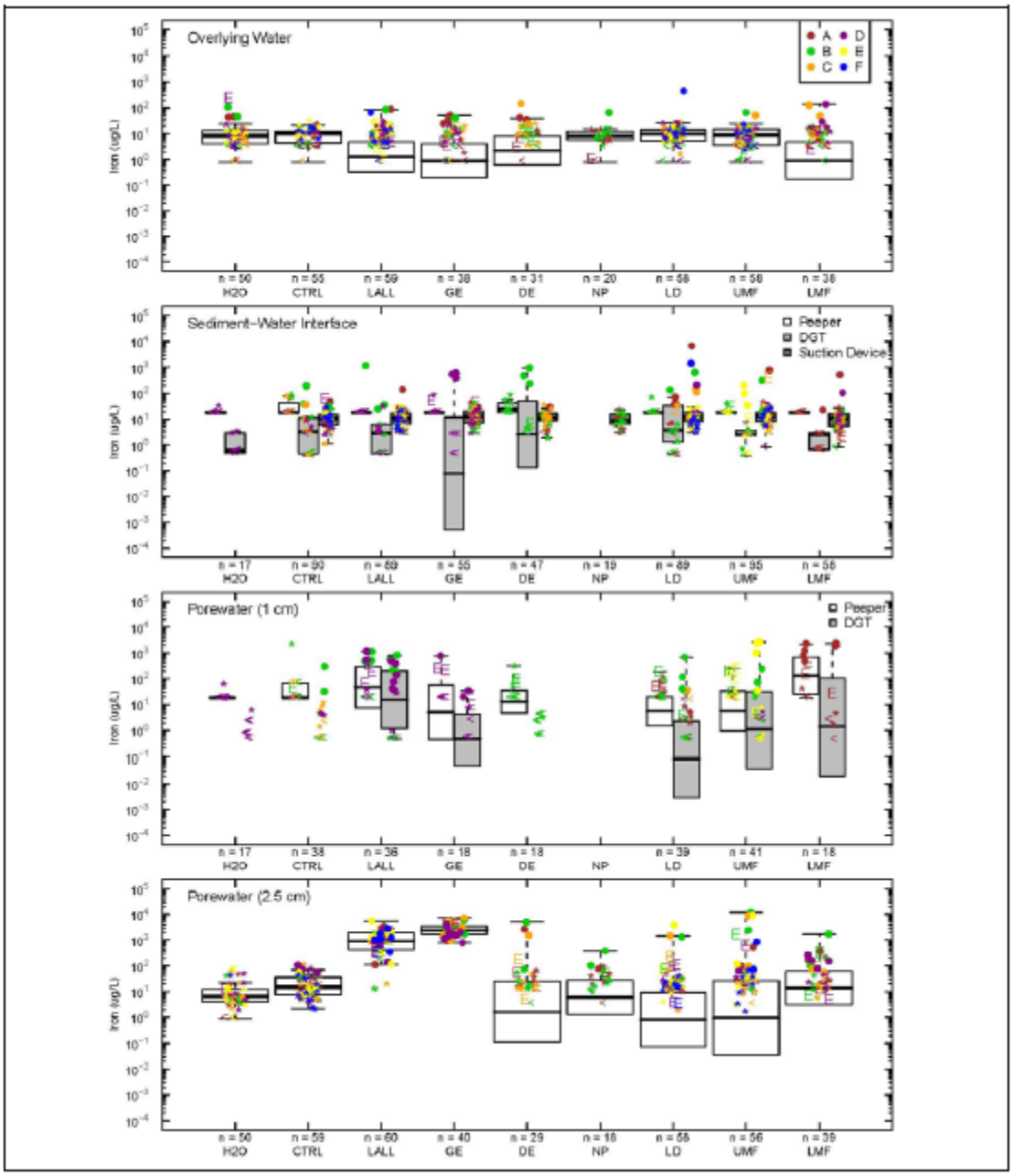


Figure D4.8. Concentrations of dissolved iron as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

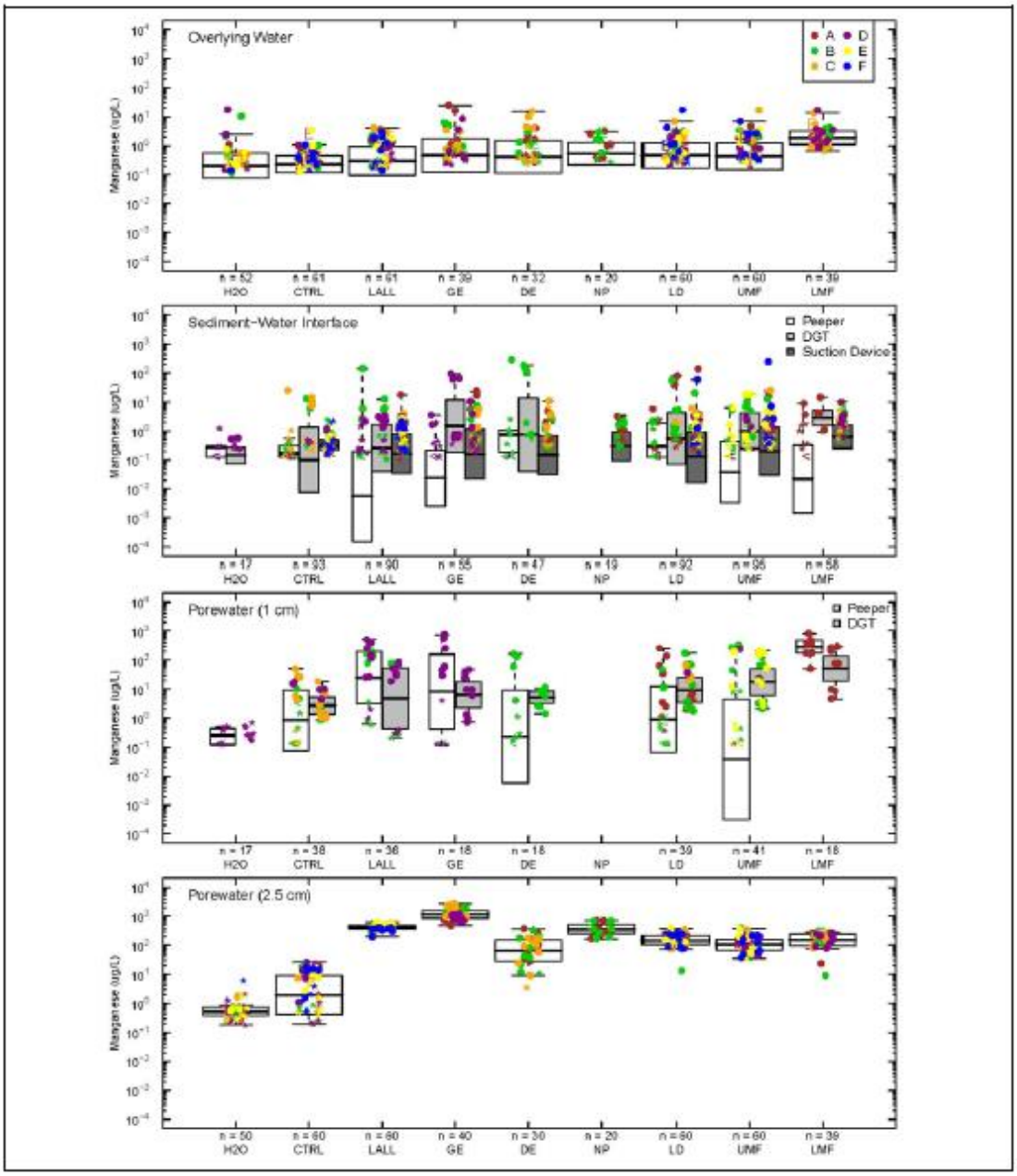


Figure D4.9. Concentrations of dissolved manganese as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

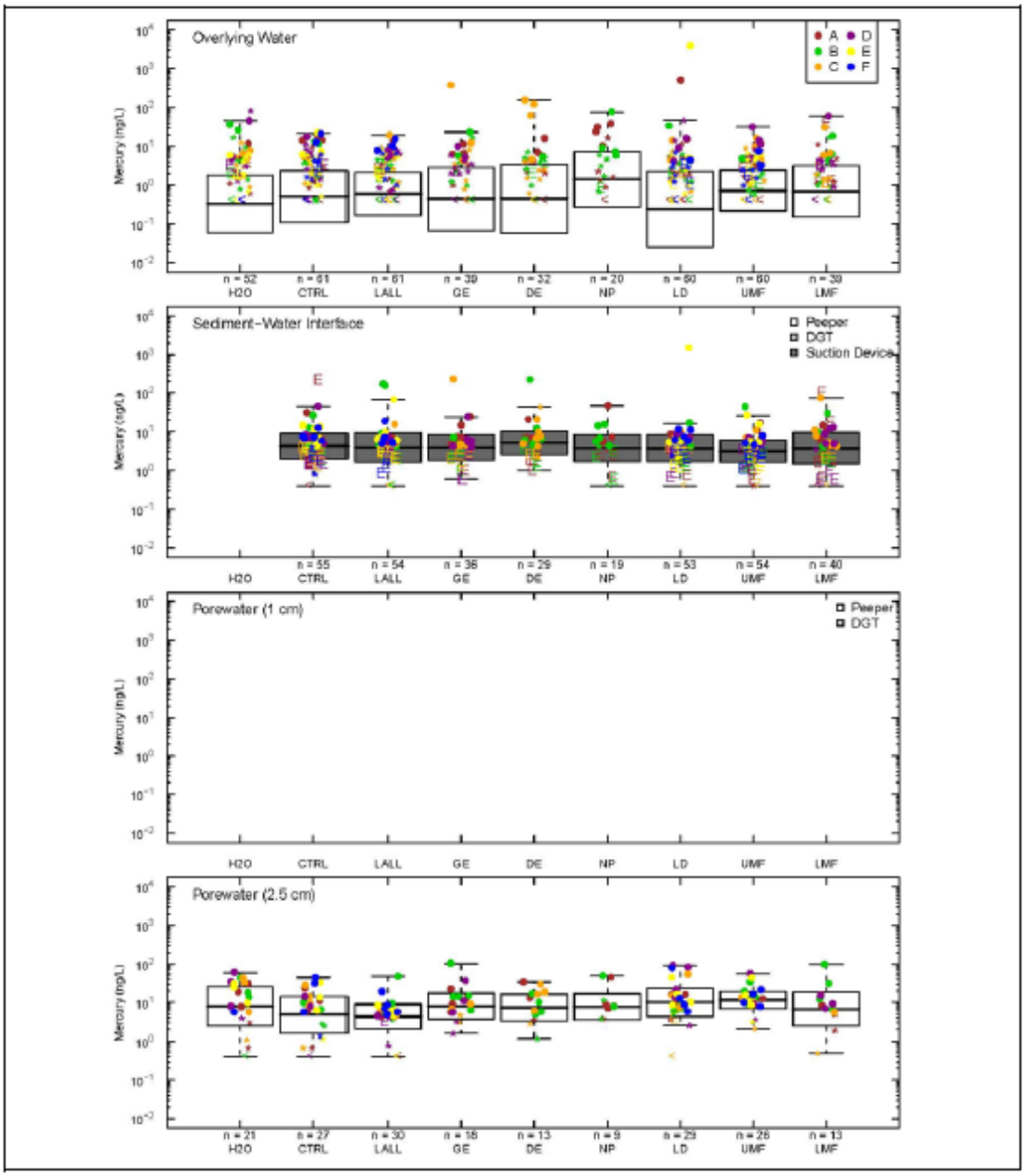


Figure D4.10. Concentrations of dissolved mercury as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

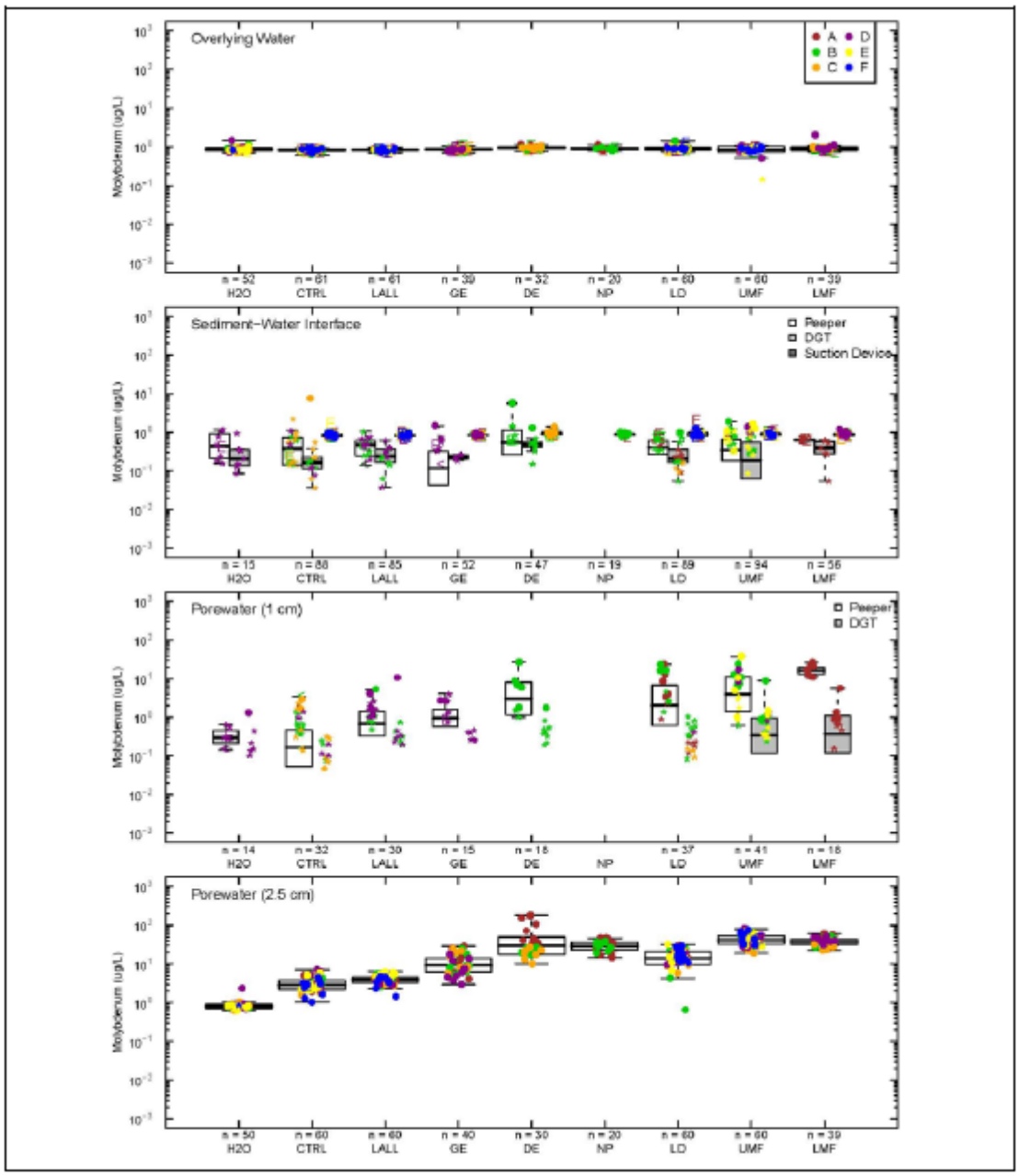


Figure D4.11. Concentrations of dissolved molybdenum as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

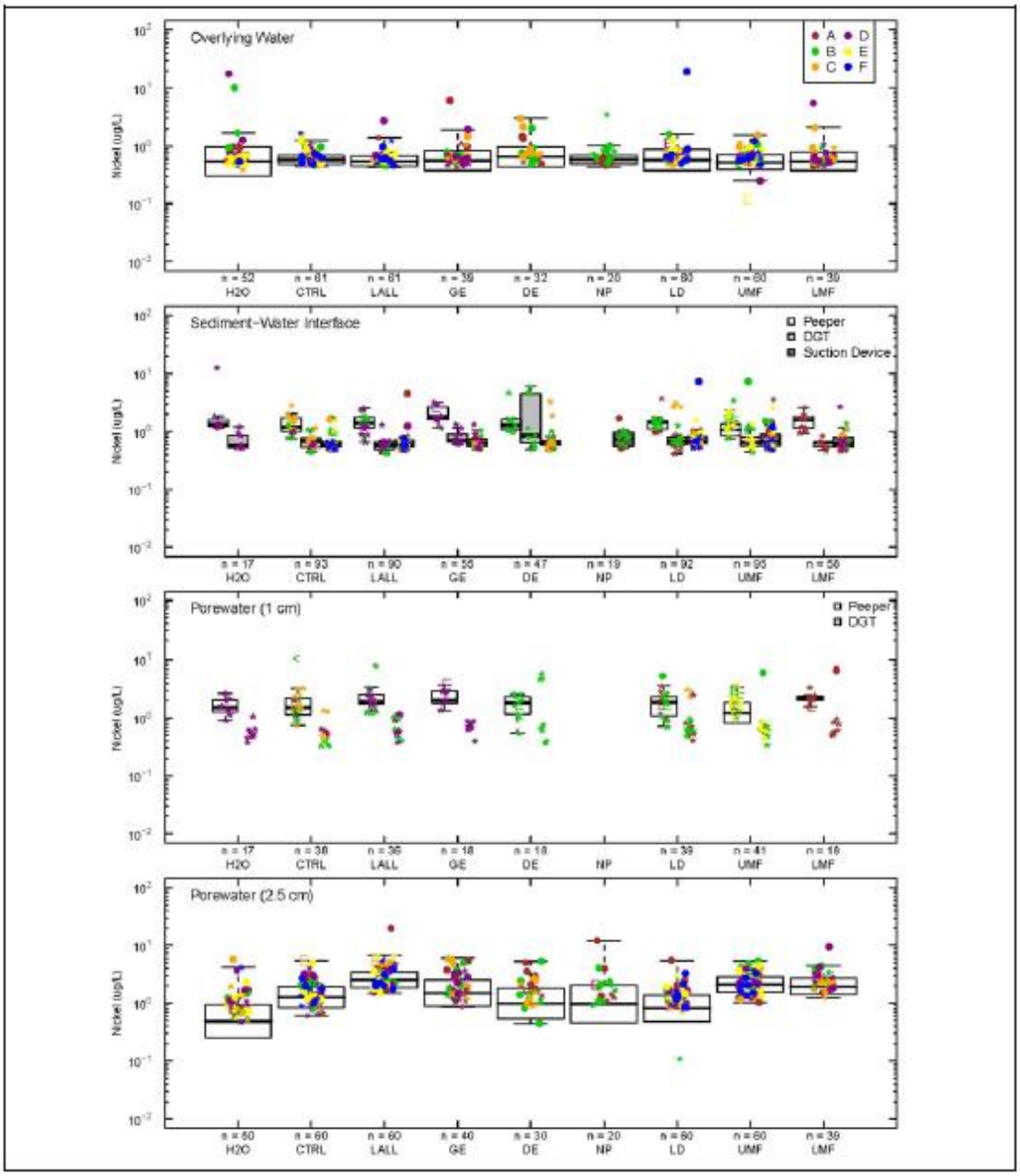


Figure D4.12. Concentrations of dissolved nickel as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

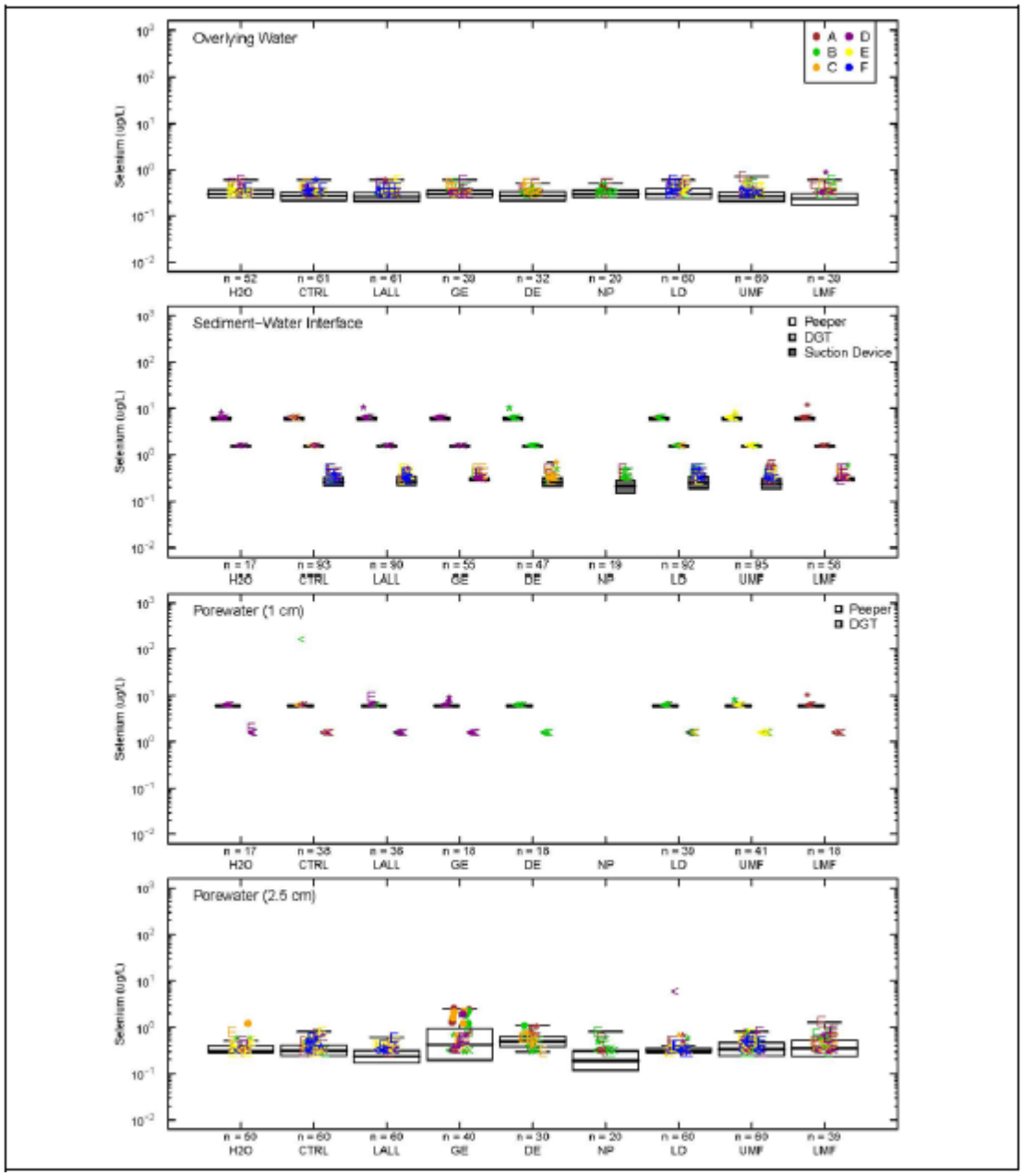


Figure D4.13. Concentrations of dissolved selenium as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

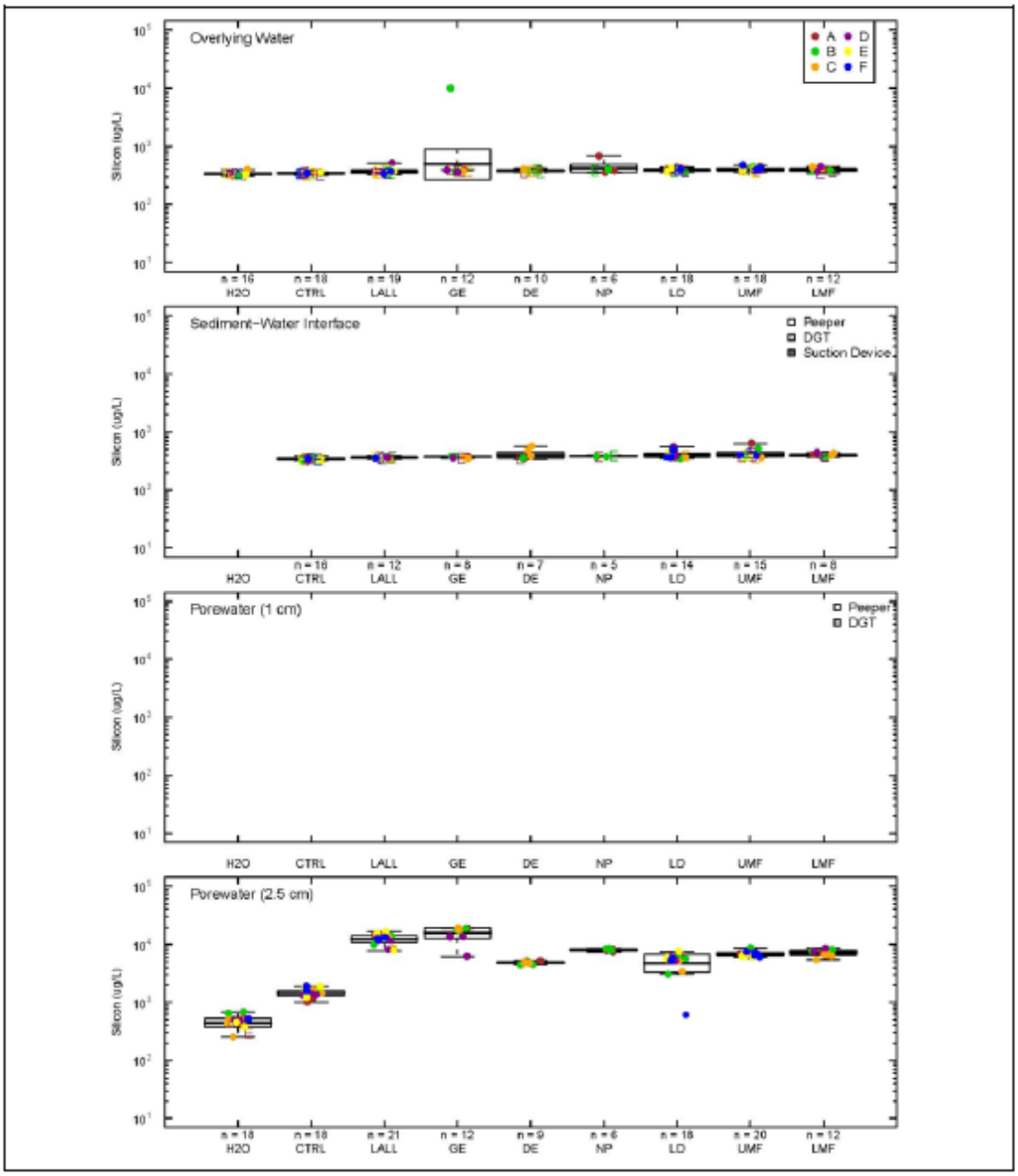


Figure D4.14. Concentrations of dissolved silicon as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

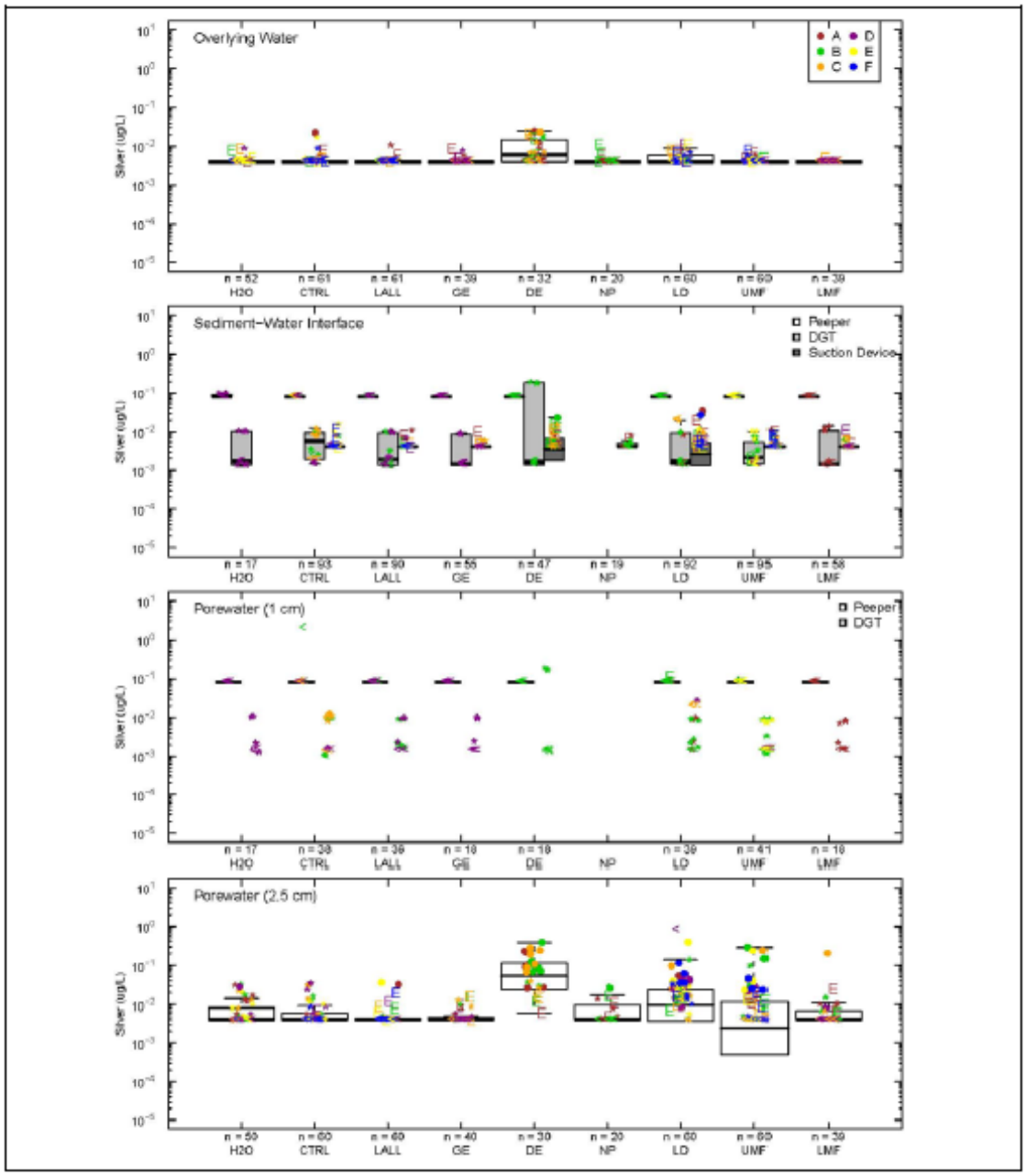


Figure D4.15. Concentrations of dissolved silver as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

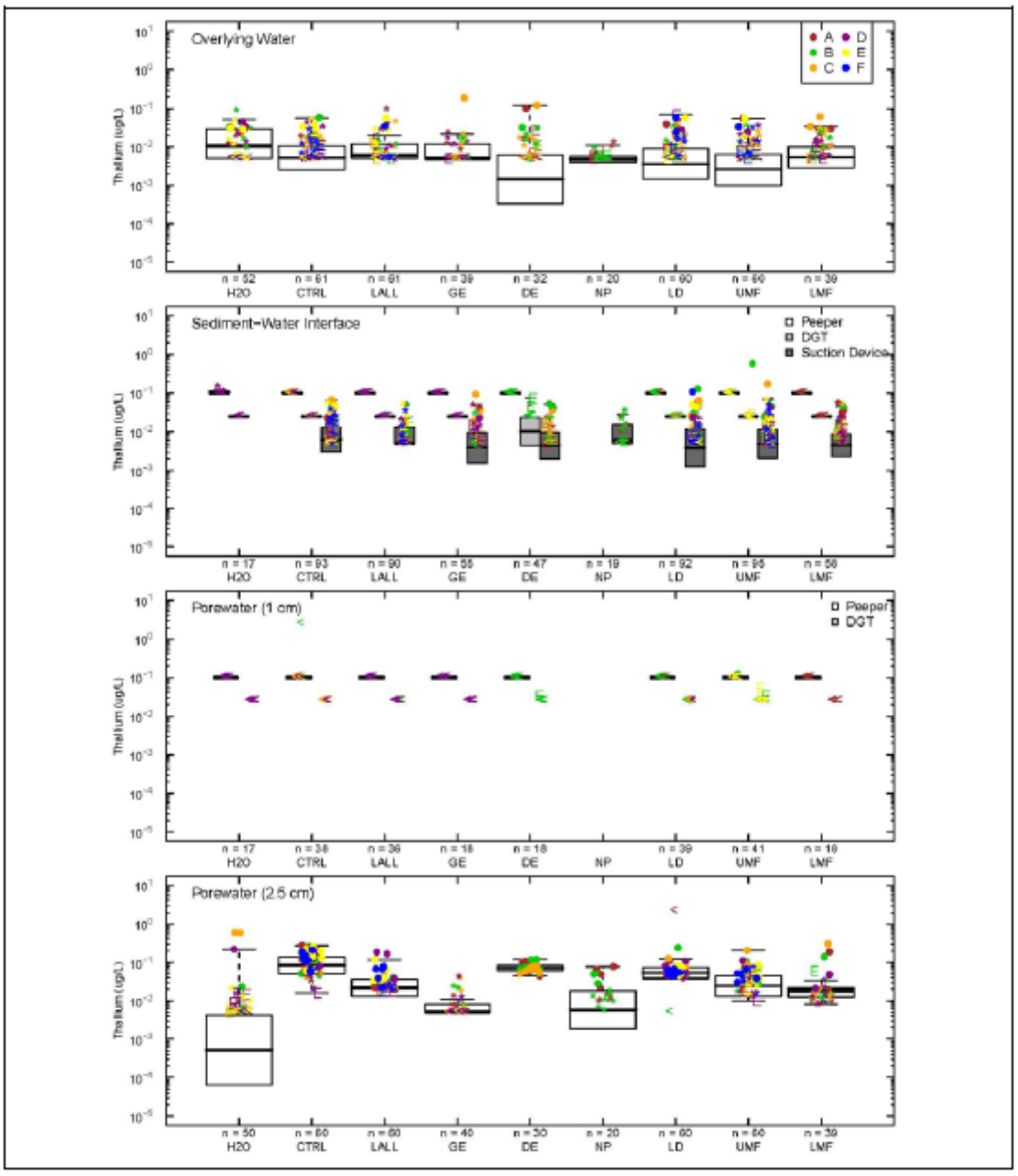


Figure D4.16. Concentrations of dissolved thalium as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

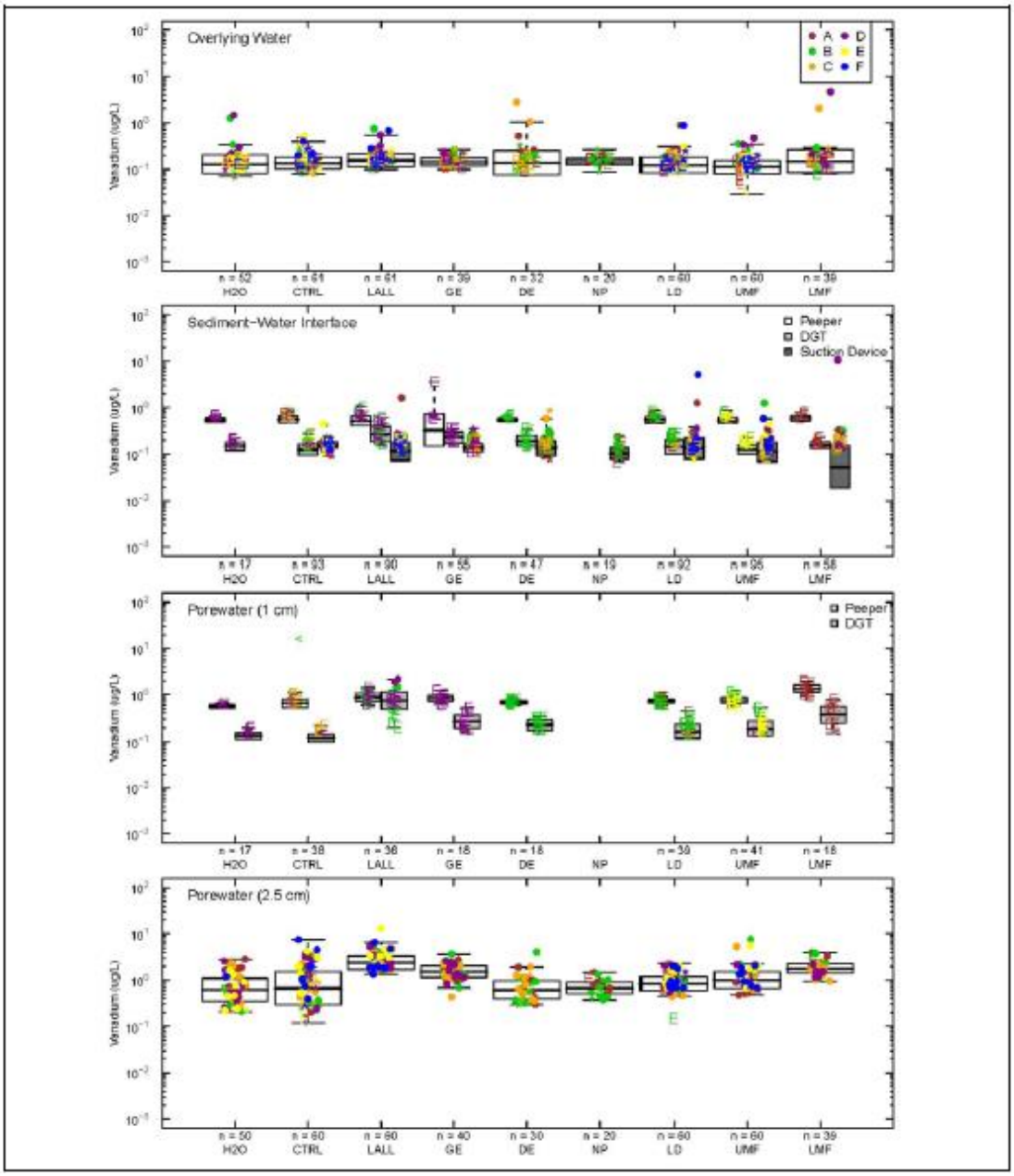


Figure D4.17. Concentrations of dissolved vanadium as a function of treatment and sample type evaluated within the white sturgeon sediment toxicity tests.

Samples types are presented in the following order: overlying water (top panel), sediment water interface water (2nd panel), pore water at 1 cm (3rd panel), and pore water at 2.5 cm (bottom panel). Where appropriate and applicable, concentrations as determined by use of different sampling techniques are identified within the top right-hand corner of respective panels. Individual measurements are shown as circles, measurements below detection are illustrated with a less than symbol ("<") plotted at the detection limit, qualified samples due to blank contamination are illustrated with an asterisks (*), and date qualified as "estimated" are represented with the symbol "E" at the estimated value. The 25th and 75th centiles from the maximum likelihood estimate (MLE; see Supplemental Materials) calculated distribution is used for the edges of the box, the median is shown as a horizontal line drawn through the middle of the box. Whiskers show maximum and minimum values exclusive of extreme values. Extreme values were identified as values that were more than 1.5 times the inter-quartile range above the 75th or below the 25th centiles.

APPENDIX E

CHAPTER 6 SUPPLEMENTAL MATERALS

Toxicity assessment of metals associated with sediment in the Columbia River to early life stages of white sturgeon

Table E1. Seeding density of sturgeon in replicated exposure chambers as determined within the 48 hrs of the study.

	Exposure Chamber Replicate Designation						Summary statistics			
Treatment Group	A	B	C	D	E	F	Minimum	Maximum	Mean	Standard Deviation
Site Sediments/Substrates										
DE	93	100	74	0	0	0	74	100	89	11
LD-01	96	94	77	73	96	90	73	96	88	9
NP-03	100	93	0	0	0	0	93	100	97	4
UMF-01	134	81	100	NA	140	98	81	140	111	23
LMF-02	107	61	96	88	0	0	61	107	88	17
References										
LALL	104	99	110	105	86	116	86	116	103	9
GE	83	165	121	90	0	0	83	165	115	32
Laboratory Controls										
CTRL	79	87	88	NA	84	147	79	147	97	25
H2O	159	131	170	149	142	0	131	170	150	13

Notes:

1. Seeding density was calculated as the sum of three components.
   Component 1 = number of mortalities recorded during the course of the Study
   Component 2 = number of lost/escaped fish recorded during the Study
   Component 3 = number of fish that survived as determined at the termination of the Study
2. NA = Not Available. Due to gaps between the screen and exposure chamber wall, an unknown number of seeded fish escaped not permitting a reliable calculation.

CTRL - artificial substrate control
DE - substrates as collected above the water line from the gravel bar at DME
GE - Genelle Eddy
H20 - water-only control
LALL - Lower Arrow Lakes
LD - Little Dalles
LMF - Lower Marcus Flats
NP - Northport
UMF - Upper Marcus Flats

Table E2. Summary of statistical tests conducted with the end-of-test survival data.

Statistical Test Number	End-of-Test Survival Data Evaluated	ANOVA (p-value)	Kruskal-Wallis (p-value)
1	LALL, NP, LD, DE, UMF, LMF	0.1549	0.1373
2	GE, NP, LD, DE, UMF, LMF	0.0995	0.0562
3	LALL, NP, LD, DE, UMF (no extreme low value), LMF	0.2573	0.2179
4	GE, NP, LD, DE, UMF (no extreme low value), LMF	0.108	0.0909

Notes:

The null hypothesis tested was that there was no difference in end-of-test survival among the treatments.

DE - substrates as collected above the water line from the gravel bar at DME

DME - Deadman's Eddy

GE - Genelle Eddy

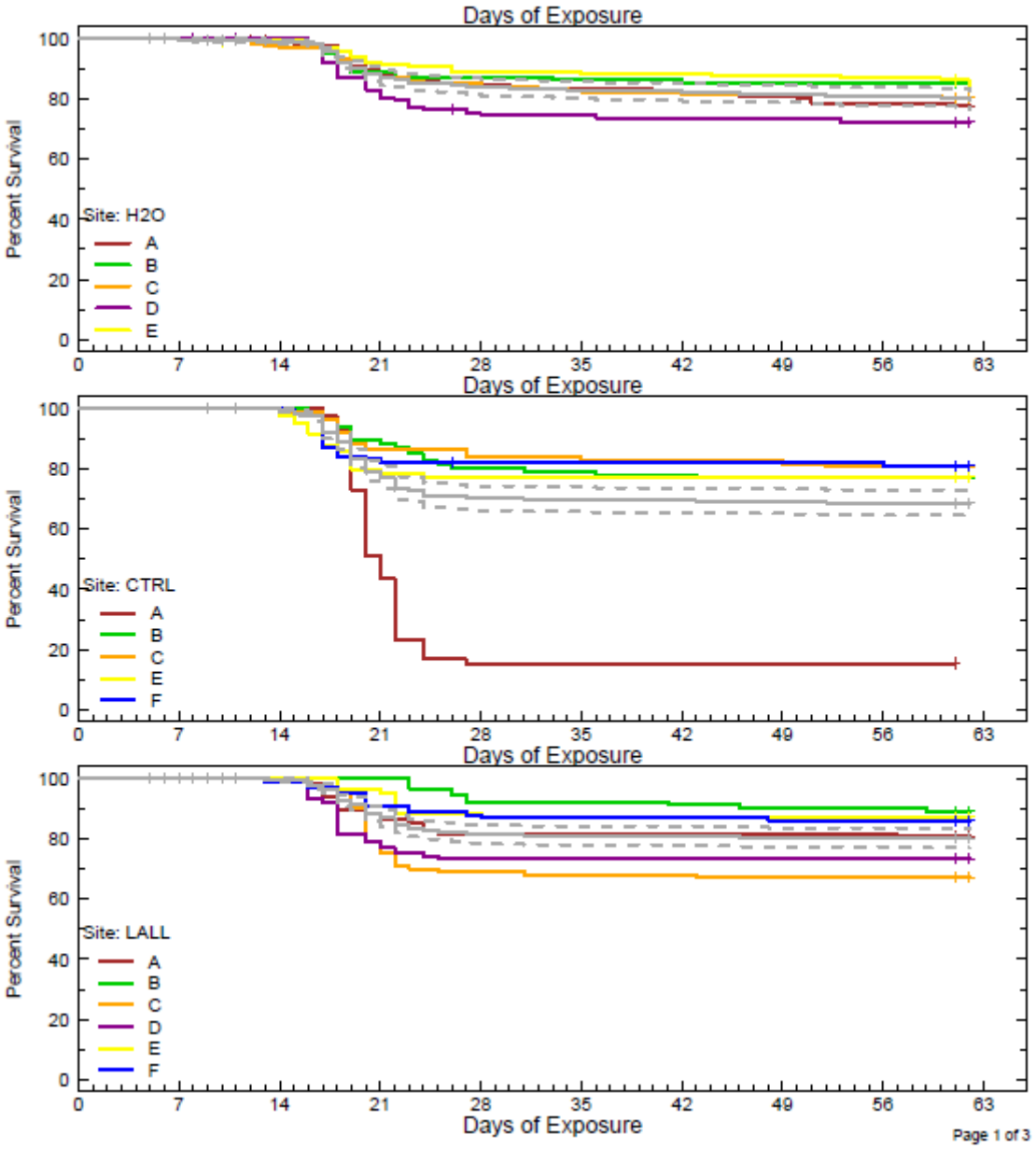
LALL - Lower Arrow Lakes

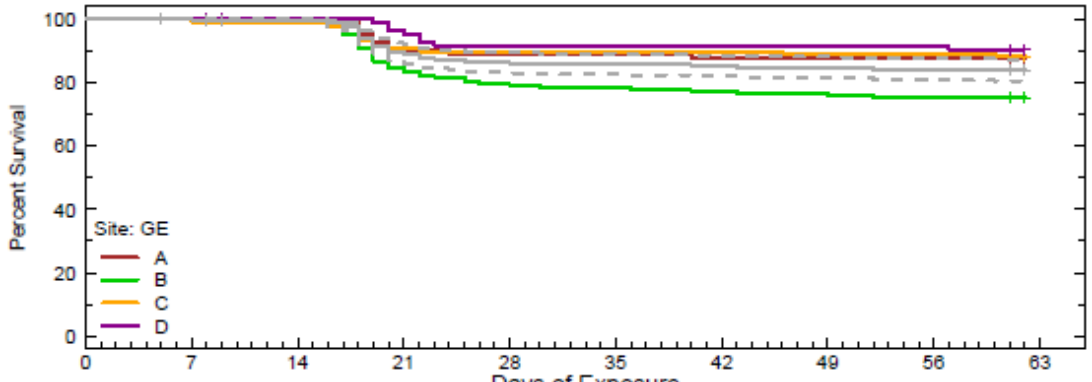
LD - Little Dalles

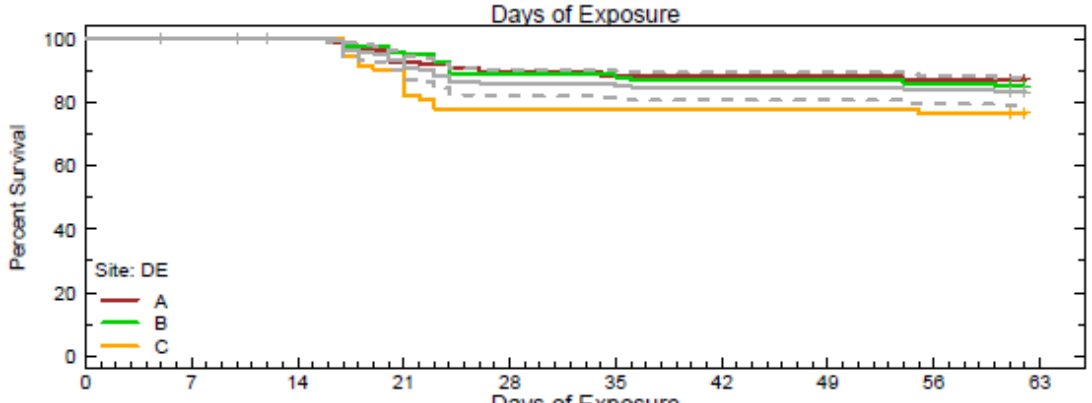
LMF - Lower Marcus Flats

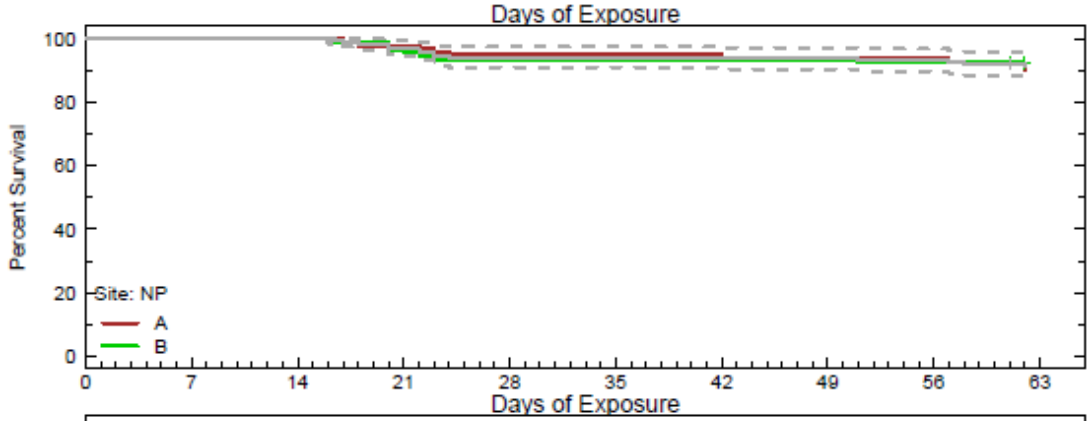
NP - Northport

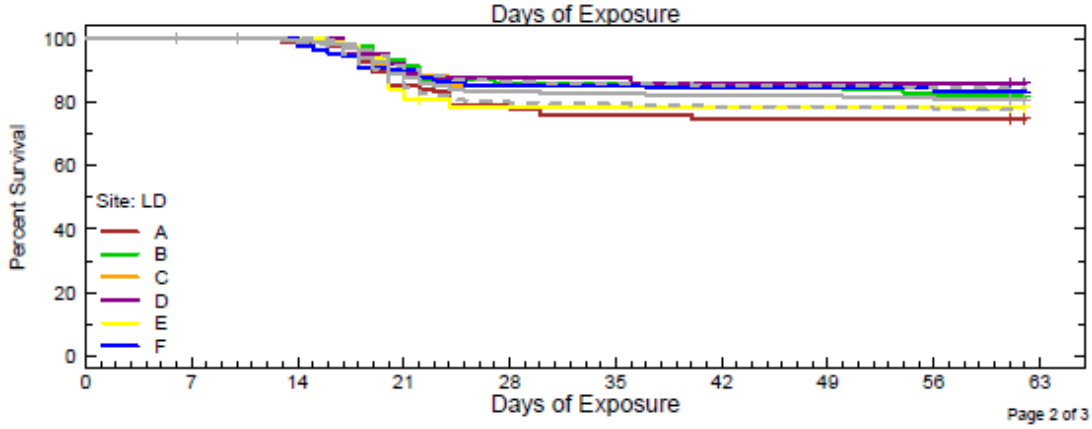
UMF - Upper Marcus Flats


Days of Exposure
Percent Survival
Site: H2O
A
B
C
D
E
Days of Exposure
Percent Survival
Site: CTRL
A
B
C
E
F
Days of Exposure
Percent Survival
Site: LALL
A
B
C
D
E
F
Days of Exposure
Page 1 of 3


Percent Survival
Site: GE
A
B
C
D
Days of Exposure


Percent Survival
Site: DE
A
B
C
Days of Exposure


Percent Survival
Site: NP
A
B
Days of Exposure


Percent Survival
Site: LD
A
B
C
D
E
F
Days of Exposure

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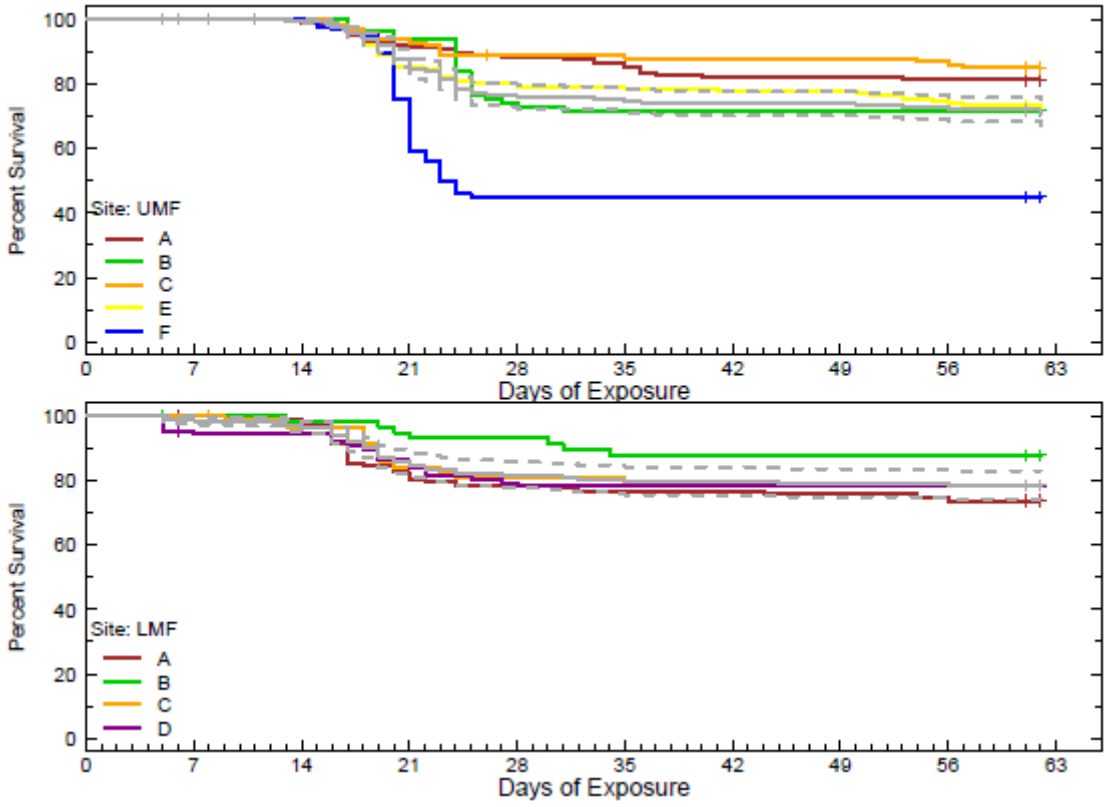

Figure E1. Survival analysis applied to sturgeon toxicity results from each treatment to give overall treatment-specific survival curves.

Replicates are represented by different colored curves. The grey curve represents the average survival for that treatment (pooled replicates), and the dashed curves represent the upper and lower 95% confidence intervals.

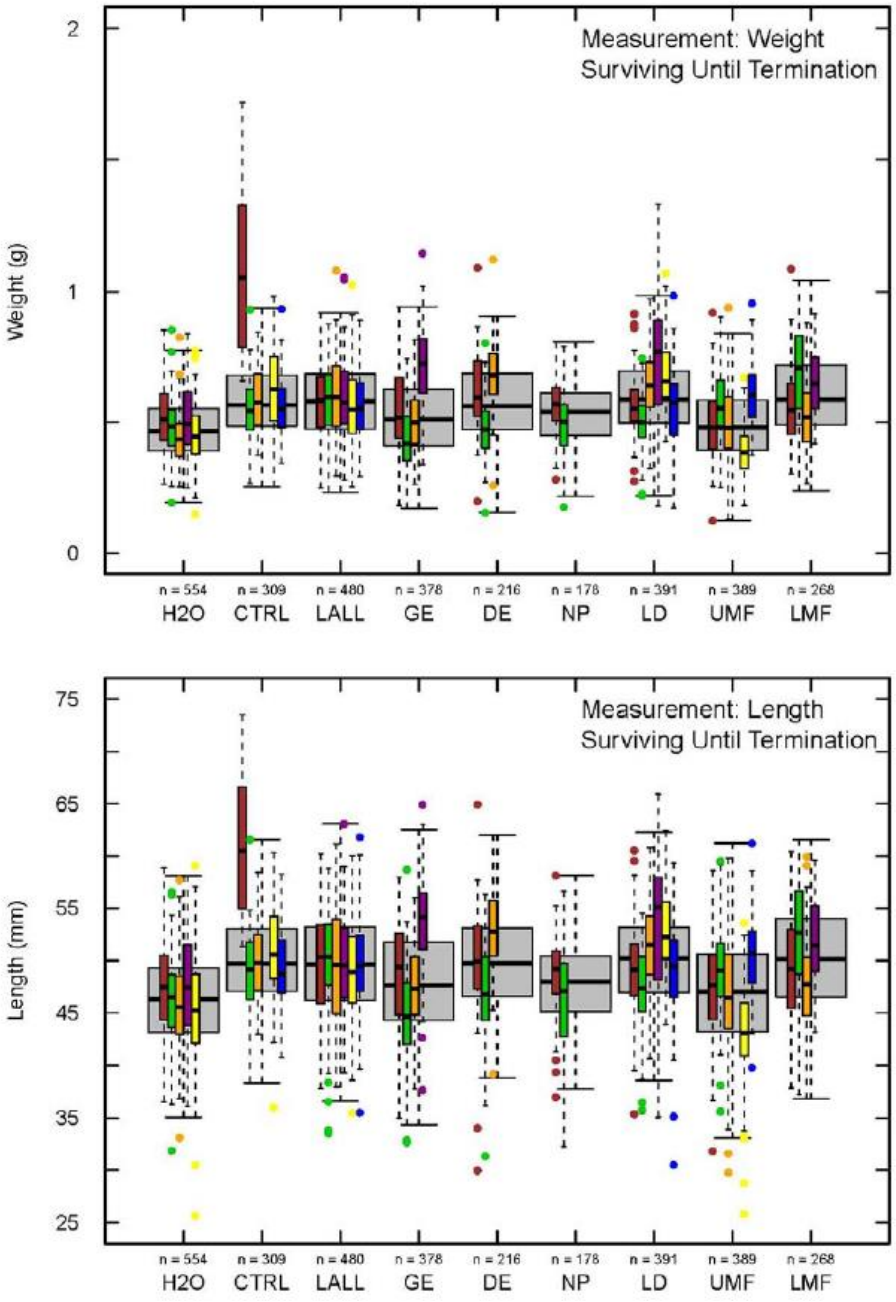

Figure E2. Growth of white sturgeon as a function of treatment at the end of the study.

For each treatment, a gray box-and-whisker plot is provided as a treatment level summary. Smaller, colored box-and-whisker plots are provided as summaries for replicate exposure chamber within a treatment. The number of fish included in length and weight calculations for each treatment is provided along the x-axis.

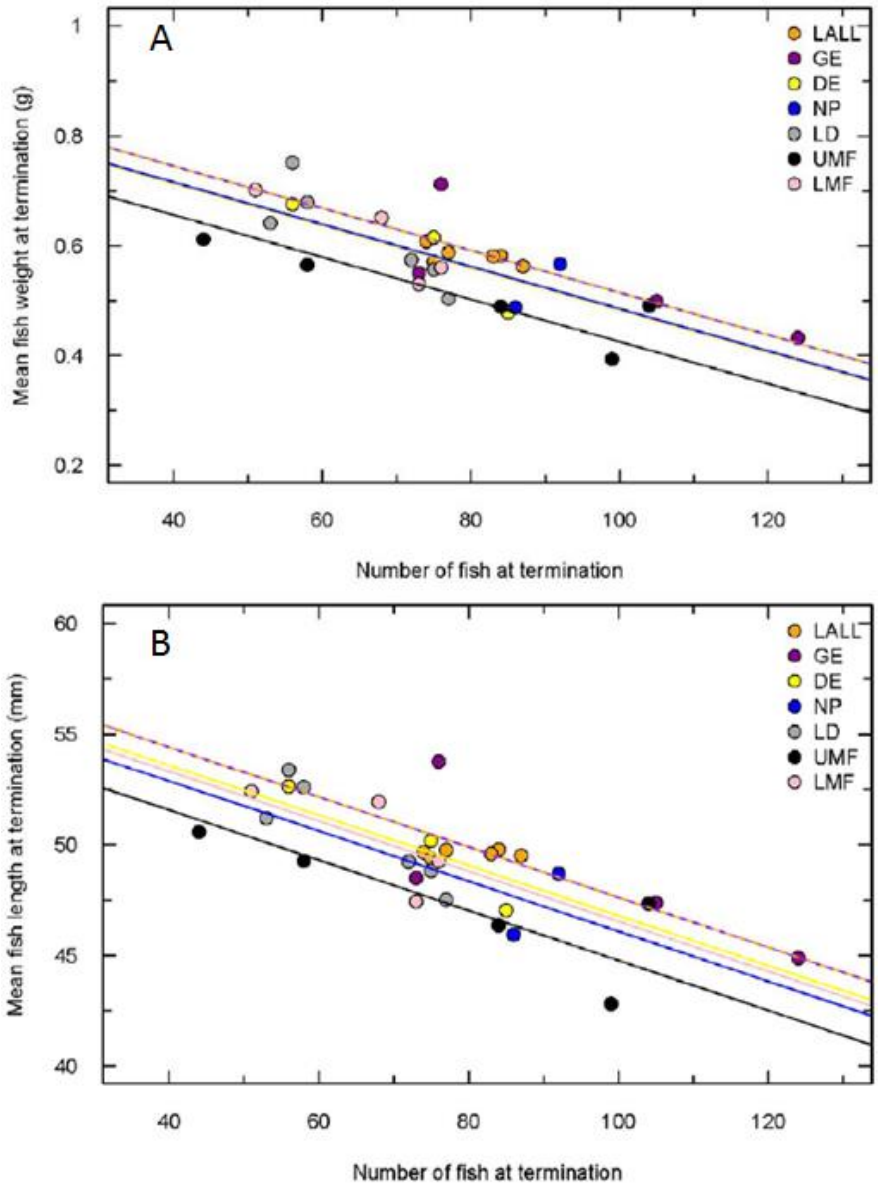

Figure E3. Average weight (A), length (B) and the number of surviving fish until study termination.

Data represent the average weight and length of fish surviving until study termination in exposure chambers. Lines represent linear predictors for each sediment treatment after application of an ANCOVA. For the ANCOVA, data form the two reference locations were pooled. The applied ANCOVA utilized a common slope assumption.

APPENDIX F

Statement of Co-authorship

The following outlines co-authorship of published articles or manuscripts that are intended for publication. All co-authors have consented to the work being included in this dissertation.

Chapter 2:

Vardy D, Oellers J, Doering J, Hollert H, Giesy J, Hecker M. 2013. *Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper.* Ecotoxicology. 22: 139-147

Vardy conducted all experiments, data analysis, and wrote the manuscript. Doering and Oellers aided in conducting specific experiments and contributed to data analysis. Giesy and Hecker were supervisors for graduate students Vardy and Doering, and Hollert was a supervisor for undergraduate student Oellers.

Chapter 3:

Vardy D, Santore R, Ryan A, Giesy J, Hecker M. 2014 a; accepted for publication. *Acute toxicity of copper, lead, cadmium, and zinc to early life stages of white sturgeon (Acipenser transmontanus) in laboratory and Columbia River water.* Environmental Science and Pollution Research.

Vardy conducted all experiments, data analysis, and wrote the manuscript. Santore and Ryan aided in data analysis involving the Biotic Ligand Model. Giesy and Hecker were supervisors for graduate student Vardy.

Chapter 4:

Tompsett A, Vardy D, Higley E, Doering J, Allan M, Liber K, Hecker M, Giesy JP. 2014. *Effects of Columbia River water on early life stages of white sturgeon (Acipenser transmontanus)*. Ecotoxicology and Environmental Safety. 101: 23-30.

For 2008 studies, Vardy designed and fabricated the experimental setup, and aided in conducting the experiments with Tompsett. For 2009 studies, Vardy designed and fabricated the experimental setup, managed the field project, and aided in conducting the experiments. Tompsett, Hecker, Higley and Vardy conducted data analysis and wrote the manuscript. Higley, Doering, and Allan aided in conducting the experiments. Liber aided in chemical analysis. Hecker and Giesy were supervisors for graduate students Vardy, Tompsett, Higley, and Doering.

Chapter 5:

Vardy D, Doering J, Ryan A, Santore R, Hecker M, Giesy J. Manuscript in preparation. *Assessment of Columbia River sediment toxicity to white sturgeon: concentrations of metals in sediment, porewater, and overlying water.*

Vardy supervised, managed and conducted all experiments, data analysis, and wrote the manuscript. Doering aided in managing and conducting the experiments. Ryan and Santore aided in data analysis. Hecker and Giesy were supervisors for graduate students Vardy and Doering.

Chapter 6:

Vardy D, Doering J, Ryan A, Santore R, Hecker M, Giesy J. Manuscript in preparation. *Toxicity assessment of metals associated with sediments from the Columbia River to early life stages of white sturgeon.*

Vardy supervised, managed and conducted all experiments, data analysis, and wrote the manuscript. Doering aided in managing and conducting the experiments. Ryan and Santore aided in data analysis. Hecker and Giesy were supervisors for graduate students Vardy and Doering.